Manuscript Details

Manuscript number	PHB_2018_754_R1
Title	Caffeine Improved Cycling Trial Performance in Mentally Fatigued Cyclists, Despite Unchanged Prefrontal Cortex Activation
Article type	Research Paper

Abstract

Purpose: To verify whether caffeine (CAF) could increase the prefrontal cortex (PFC) activation and improve 20 km cycling time trial (TT20km) performance in mentally fatigued cyclists. Methods: After preliminary TT20km, twelve recreational cyclists (VO2MAX of 58.9 ± 6.2 mL·kg·min-1) performed a familiarization with a cognitive test to induce mental fatigue (MF) and psychological scales. Thereafter, they performed: 2) a baseline TT20km; 3) a mentally fatigued TT20km (MF); 4 and 5) a mentally fatigued TT20km after CAF (MF+CAF) or placebo (MF+PLA) ingestion, in a double-blind, counterbalanced design. Performance and psychological responses were obtained throughout the TT20km, while PFC electroencephalography (EEG) theta wave was obtained before and after the mental fatigue test. Results: The mental fatigue-induced increase in EEG theta wave (↑ ~ 4.8 %) was reverted with CAF (↓ 8.8 %) and PLA ingestion (↓ 4.8 %). CAF improved TT20km performance in mentally fatigued cyclists by reducing time ($p = 0.00$; ↓ ~1.7 %) and increasing WMEAN ($p = 0.00$; ↑ ~3.6%), when compared to MF+PLA. The RPE-power output ratio was lower ($p = 0.01$), but affect ($p = 0.018$), motivation ($p = 0.033$) and emotional arousal ($p = 0.001$) were greater throughout the TT20km in MF+CAF than in MF+PLA. Conclusions: CAF ingestion improved TT20km performance and psychological responses in mentally fatigued cyclists, despite the unaltered PFC activation.

Keywords	Fatigue; Placebo; RPE; Pacing; Supplementation

Corresponding Author	Flávio Oliveira Pires
Corresponding Author's Institution	School of Arts, Science and Humanities - University of Sao Paulo
Order of Authors	Paulo Estevão Franco, Cayque Brietzke, Raul Canestri, Márcio Goethel, Florentina Hettinga, Tony Meireles Santos, Flávio Oliveira Pires
Suggested reviewers	Andrew Renfree, Ben Rattray, Andrew Edwards, Eduardo Penna

Submission Files Included in this PDF

File Name [File Type]

Estevão-Alvarenga et al (Cover letter).docx [Cover Letter]

FRANCO-Alvarenga et al (Point-by-point letter R1).docx [Response to Reviewers]

FRANCO-Alvarenga et al (Manuscript R1).docx [Revised Manuscript with Changes Marked]

FRANCO-Alvarenga et al (Bullet-Points).docx [Highlights]

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Figure 1.tif [Figure]

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

COVER LETTER

We would like to submit the manuscript entitled "**Caffeine Reverted Mental Fatigue-Reduced Cycling Trial Performance Despite Unchanged Prefrontal Cortex Activation**" to be analyzed by the Editorial Board of the Physiology and Behavior, as a research article to the Special Issue "Psychophysiological and behaviour evaluation of human performance".

The authors state that the manuscript contains only original material that has not previously been published, and is not currently under consideration elsewhere, nor will be submitted elsewhere until a final decision has been made by the journal. All of the experimental procedures were approved by the Institutional Ethics Committee (University of São Paulo) and conformed to the Declaration of Helsinki. The experimental procedures were explained to the participants before they signed an informed consent form.

Corresponding Author

FLÁVIO O PIRES (✉)

School of Arts, Science and Humanities, University of São Paulo, Brazil

Arlindo Bettio Avenue, 1000, 03828-000. Tel: 55+11+3091-8157

E-mail: *piresfo@usp.br*

Dear Dr. Vicente Javier Clemente Suárez, Editor, Physiology and Behavior;

Regarding our manuscript PHB_2018_754, we would like to thank you for the opportunity to resubmit the manuscript. The reviewers have addressed important questions that contributed to improve the document. We have worked to incorporate all possible/relevant suggestions, as well as to provide detailed justification of crucial points. Thus, modifications in the revised manuscript were highlighted in red and a point-by-point letter, explaining every raised point is presented below. We hope we have attained the required standard of the journal.

Comments from the editors and reviewers:

-Reviewer 1

- General

This manuscript reports the results of a well-designed double blind cross-over experiment to test if caffeine can alleviate mental fatigue impairment of time trial performance in trained cyclists. The result suggest this to be case. The paper is very well written and presents the results in a clear way.

Reply: Thank you very much for your review and encouraging words.

I only have some minor comments:

Point 5 of the bullet points is not clear to me.

Reply: Thanks, we reworded it.

L240, mention that these are means and SDs.

Replay: Thanks for the comment, we included.

L291, 'performed'.

Reply: Thanks, we corrected this.

L365 and 367, not certain that the word 'manipulated' is the right one here, what about 'manufactured'? Also, it is not clear from this description if CAF and PLA were looking similar or not.

Reply: Thanks, we corrected this replacing “manipulated” by “formulated” and including they were similar in appearance.”

L567, the phrase on p values needs to be rewritten differently.

Reply: Thanks, we reworded this phrase.

L959, you can cite a recent paper that precisely looked at dosage and peripheral effects: https://www.ncbi.nlm.nih.gov/pubmed/30566390

Reply: Thank you, we inserted this reference.

Figure 2 is not helpful, can be left out.

Reply: Removed, accordingly.

-Reviewer 2

- Thank you for the opportunity to review the manuscript "Caffeine reverted mental fatigue-reduced cycling trial performance despite unchanged prefrontal cortex activation". The authors have conducted a study in which a placebo-controlled cross-over study was used to identify if caffeine could improve performance after a mental exertion task. Additionally, the authors sought to investigate if changes in EEG activity, and other mechanistic measures, were altered with caffeine. The study was enjoyable to read and promising to make an important contribution to the area. However, at present I cannot understand the analysis, nor results section.

Reply: thank you very much for your careful review.

Major comments:

It is quite interesting that some reliability data has been reported in this study. Note however that familiarisation trials are often conducted in studies, and thus the ideal reliability data would come from the comparison of more trials. If the participants were informed that trial 1 was a familarisation, this may also influence the reliability. It is impressive that the cyclists were as reliable as they were given all of this, I note they engage in a reasonable volume of training and competition, and as such the reliability data is appropriate within this context - well-practised cyclists comparing 2 trials with no prior familiarisation.

Reply: We understand your concern. We would like to point out that no specific information were given to cyclists about familiarization. As described in the "Study design" section, the preliminary trial (trial 1) was used to identify eligible cyclists (those who completed the 20 km within 33 min) - potential participants were informed that only cyclists attaining a time threshold would be eligible (time threshold was revealed only after the study completed - TT20km finished within 33 min). Please, note that the word "familiarization" was referring to the cognitive test (RVIP test), which was performed by eligible cyclists after the preliminary trial. Furthermore, eligible cyclists were informed (when they signed the informed consent form) that the study had 5 visits (two TT20km without manipulation, three including cognitive task with CAF/or/PLA/or no supplement), thus no mention about "familiarization" was needed. Importantly, performance responses (time and Wmean) showed a typical error of measurement (trial 1 vs trial 2) comparable to those reported by a study designed to verify the reproducibility of 10 and 20 km time trial performance in cyclists [1]. Additionally, studies have reported strong reliability in performance measures obtained in cycling time trials with different distances [1–3], so that considering the performance criteria used to homogenize the sample, the typical error of measurement of 0.8 % (time) and 3.1 % (Wmean) was expected. In contrast, a placebo-controlled cross over design including reliability performance measures before caffeine ingestion is unusual in the exercise sciences literature. We performed amended the "study design" description in order to make it clearer. Please, let us know if it is ok.

D.N. Borg, J.O. Osborne, I.B. Stewart, J.T. Costello, J.N.L. Sims, G.M. Minett, The reproducibility of 10 and 20km time trial cycling performance in recreational cyclists, runners and team sport athletes., J. Sci. Med. Sport. 21 (2018) 858–863. doi:10.1016/j.jsams.2018.01.004.

B.L.M. Smits, R.C.J. Polman, B. Otten, G.-J. Pepping, F.J. Hettinga, Cycling in the Absence of Task-Related Feedback: Effects on Pacing and Performance, Front. Physiol. 7 (2016) 348. doi:10.3389/fphys.2016.00348.

C.A. Williams, S.D. Bailey, A.R. Mauger, External exercise information provides no immediate additional performance benefit to untrained individuals in time trial cycling., Br. J. Sports Med. 46 (2012) 49–53. doi:10.1136/bjsports-2011-090257.

Unfortunately I don't feel the statistics/results are clear as to what was carried out, and I have a few concerns with it as presented. Firstly, it appears as though comparisons between the MF trials (placebo and caffeine) were made with the baseline (trial 2 - or the second familiarisation) trial - lines 304-306. But the results presented, such as in the "proof-of-concept" results section, suggest that a paired t-test was used to compare between placebo and caffeine. I am not sure what is compared. It should be placebo v caffeine when investigating the effect of caffeine. In some of the results in this section, the means also look quite similar, especially considering the large relative SDs, but the p values and effect sizes reported seem strongly significant with large effect sizes. The stats just don't look right here. Further, there are a large number of paired t-tests being carried out, this seems quite inappropriate providing high probability that there are type 1 errors. I suggest alternate statistical methods be sought, or p-values are adjusted across many more measures than just the mixed model analysis carried out. cannot understand what statistics have been carried out. Given the concerns and confusion I have around the statistics and results, I have not read further than this section.

Reply: Sorry, actually your concern made us to realize that we should have been clearer when detailing our design and comparisons. Thanks for drawing attention to this point. We designed the three first sessions to provide a strong, adequate control before the investigation of CAF effects on mentally fatigued cyclists. Thus, we performed the three first sessions in sequential order, as we aimed to measure the TT20km reliability (trial 1 vs trial 2; preliminary trial vs baseline trial) before performing a proof-of-concept study of mental fatigue effects (trial 2 vs trial 3; baseline trial vs MF trial). In this regard, despite being extensively investigated for the last ~10 years, only one study investigated MF effects on TT20km performance. Importantly, as MF reviews [4,5] have suggested that MF effects on performance may depend on the type of exercise under consideration (open vs closed-loop exercise, endurance vs high-intensity exercise), we decided to confirm (proof-of-concept) rather than assume that MF impaired TT20km performance. Thus, after the proof-of-concept trials, we investigated the study of CAF effects on cycling performed in mentally fatigued cyclists, having MF+CAF and MF+PLA trials performed in counterbalanced order. Therefore, we used paired t-tests within each phase of the study (i.e. reliability; proof-of-concept; CAF effects), thus controlling the type I error (as we did not perform multiple t-tests between > trials, no correction is needed). Regarding the mixed model comparisons, this analysis was carried out in a particular dataset that

involved a repeated-measures design, that is, when we compared variables obtained during the trials in MF+CAF and MF+PLA. We have rephrased "study design" and "statistical analysis" sections; please let us know if it is ok.

J. Van Cutsem, K. De Pauw, L. Buyse, S. Marcora, R. Meeusen, B. Roelands, Effects of Mental Fatigue on Endurance Performance in the Heat, Med. Sci. Sport. Exerc. 49 (2017) 1677–1687. doi:10.1249/MSS.0000000000001263.

K. Martin, R. Meeusen, K.G. Thompson, R. Keegan, B. Rattray, Mental Fatigue Impairs Endurance Performance: A Physiological Explanation, Sport. Med. 48 (2018) 2041–2051. doi:10.1007/s40279-018-0946-9.

Specific comments:

Introduction

Line 122-123: the first line says there "may" be a difference (and includes reference 3), but then the following sentence states that reference 3 verifies the fact. I think it would be better to say that mental fatigue "can" impair.... and in the next sentence talk about how study 3 supports this. The use of verify should be changed throughout the manuscript as I don't believe any particular study unconditionally confirms any result.

Reply: Mental fatigue affects most endurance exercises, in particular. For example, maximal strength, power and anaerobic exercises have been unaffected by mental fatigue. Therefore, mental fatigue effects on physical performance may/may not be present (thus the use of "may" as auxiliary is preferable, given we are talking about possibility rather than ability). We agree with you that no particular study unconditionally confirms a theory (we think you meant "theory" rather than "results"), regardless the semantic choice to express it. That is why we prefer the use of "may" instead of "can". We have reworded some parts; please see if it is ok.

Line 140-146: I don't think there is evidence that this sequence of events has to occur for mental fatigue to have its effect, indeed, i imagine some of them are just related events. suggest re-working

Reply: According to decision-making/goal-driven theories applied to exercise performance scenarios, the mental sense of effort simultaneously mediates two conflicting cognitive/behavioral processes that indicates utility (i.e. reward) and disutility (i.e. cost), respectively, the incentive-performance and effort-performance relationship. It

has been shown good internal consistency, reliability and concurrent validity between an increased cost-benefit thinking and a deliberative mindset shift towards goal-disengagement processes [6]. Furthermore, it has been shown that mental fatigue may increase the cost-benefit thinking [7], thus influencing the conflicting cognitive/behavioral processes during exercise that leads to performance impairments (premature exercise disengagement). In this regard, the decision process of continuing or disengage from an exercise is accomplished through the higher-order cognitive control (encoding and storage relevant cues, using working and long-term memory). We rationalized this in lines 140-146. However, we agreed that the inclusion of "feeling of tiredness" may be "lightly speculative" in this context so that we rephrased this part; thanks!

A. Venhorst, D. Micklewright, T.D. Noakes, Towards a three-dimensional framework of centrally regulated and goal-directed exercise behaviour: a narrative review, Br J Sport. Med. (2017). doi:10.1136/bjsports-2016-096907.

M.A. Boksem, M. Tops, Mental fatigue: costs and benefits, Brain Res Rev. 59 (2008) 125–139. doi:10.1016/j.brainresrev.2008.07.001.

Line 146: "cost-future reward relationship" i think this is referring to the concept of effort discounting. As the wording is, I don't think it is clear what is meant here.

Reply: We wrote this to make it clear. Please, let us know if it is ok.

Line 167: should it be clarified that the higher RPE than expected is in reference to when power output or speed is known. RPE by itself does not provide useful performance feedback.

Reply: Clarified, accordingly.

Line 180: strict-life style in daily activities - is this referencing that recreational athletes typically have busy lives whose time cannot be dedicated to recovery when not training? I think this should be a little clearer.

Reply: Included, accordingly (encompassing dietary restrictions, longer work-days, reduced time for recovery and limitations to social life).

Methods:

Line 283: wording unclear, suggest something like cyclists attended the laboratory on four more occasions

Reply: Clarified, accordingly.

Line 298: the use of the term "proof-of-concept" is confusing. I am unclear what is meant here. Mental fatigue influencing performance is well established, so there is no proof of concept there. This sentence is also not clear.

Reply: This is an unusual, well-controlled study as we presented reliability and proof-of-concept data, before investigating caffeine effects on TT20km. We have answered this question above, please let me know if it is ok.

Line 338: should the units in which a.u. is used be "counts"

Reply: Clarified, accordingly.

Line 382: should there be a minor re-wording? - "that is, they were blinded...."

Reply: Changed, accordingly.

Line 428: where each of these time-points measured for 3 min? I think so, but please clarify.

Reply: Sorry, we did not understand what you mean. We have tried to clarify it, anyway.

Line 462: analogue – spelling

Reply: Thanks for your correction.

Line 464: perhaps a translation issue, I'm not sure, but would it be truer/more correct to say that the zero scale was anchored by "no mental fatigue"?

Reply: Thanks for your comment, we used anchors similar to a previous mental fatigue study by Smith et al., [8]. We have amended it.

M.R. Smith, L. Zeuwts, M. Lenoir, N. Hens, L.M. De Jong, A.J. Coutts, Mental fatigue impairs soccer-specific decision-making skill, J Sport. Sci. 34 (2016) 1297–1304. doi:10.1080/02640414.2016.1156241

Line 479: similar to above, should this be "not at all motivated"

Reply: We used anchors similar to a previous mental fatigue study by Salam et al., [9].

H. Salam, S.M. Marcora, J.G. Hopker, The effect of mental fatigue on critical power during cycling exercise, Eur. J. Appl. Physiol. 118 (2018) 85–92. doi:10.1007/s00421-017-3747-1

-Reviewer 3

-General:

In my opinion, the manuscript entitled "Caffeine reverted mental fatigue-reduced cycling trial performance despite unchanged prefrontal cortex activation" presents a good, strong, controlled and reliable method to properly answer the research question. In general, the manuscript is well written, and the results are well described. Although, in my opinion, there is some methodological misinterpretation on how data was organized for analysis and this may have led to a misunderstanding discussion of the results.

My main concern is regarding the non-inclusion of the baseline data (the control situation without MF presence) in performance and psychological comparisons. Without this data, the interpretation of the results could be limited. As comparisons have been made, we can only conclude that acute caffeine intake is superior to placebo, however, it has not been shown whether it can counteract the negative effects of MF. This statement could only be true if the acute caffeine ingestion is capable of "return" the performance to a similar level of the condition without MF. The way that the manuscript was conducted lead to the idea of the ecological validity of the caffeine ingestion. I suggest including the baseline in comparisons or change the article's approach.

Reply: Thank you very much for your careful review. Regarding your main concern involving baseline comparisons, we think our writing approach should have been better, thus allowing a clear interpretation of the results. Sorry! We have rephrased "study design" and "statistical analysis" sections in order to improve the readers' comprehension. Briefly, we performed the three first sessions in sequential order, as we aimed to measure the TT20km reliability (trial 1 vs trial 2; preliminary trial vs baseline trial) before performing a proof-of-concept study of mental fatigue effects (trial 2 vs trial 3; baseline trial vs MF trial). Thus, thereafter we investigated the study of CAF effects on cycling performed in mentally fatigued cyclists, having MF+CAF and MF+PLA trials performed in counterbalanced order. Therefore, we had baseline and MF trials being performed in sequential order, before MF+CAF and MF+PLA trials. In order to not strike methodological assumptions such as the design balancing and ordering, we did not perform multiple comparisons involving MF+CAF, MF+PLA, MF and baseline trials

together, as we could have not ensured the due balanced order of all conditions (i.e. baseline, MF, MF+CAF and MF+PLA) when carrying out ANOVA/mixed models comparisons. Instead, we carried out a paired t-test to investigate the effects of mental fatigue on TT20km performance (i.e. proof-of-concept) and, thereafter, a paired t-test to verify if caffeine improved TT20km performance in mentally fatigued individuals. Thus, in strict terms you are right, we cannot assume that caffeine reverted mental fatigue-reduced performance, instead we can assume that caffeine improves TT20km performance in mentally fatigued cyclists. In contrast, we can assume CAF revert MF effects on PFC activation as comparisons of pre-to-post RVIP test EEG measures were used. We have reworded it throughout the manuscript, thus given more accuracy to our assumptions. Importantly, to mitigate inter-individual data variability we expressed MF+CAF and MF+PLA performance as delta alterations from MF TT20km, as reported elsewhere [10]. Please, let me know if it is ok.

Below I present my specific considerations for each section.

Bullet points:

1 – By the results of the manuscript, I think the first bullet point can be direct: MF impair performance.

4- Which alterations and their directions (increase or decrease motivation, for example).

5- By reading the abstract, the 5th bullet point is general and does not add valid information.

Reply: Rephrased, accordingly.

Introduction

The introduction is very well written and conducts the reader to the specific objectives of the study.

Lines 191-196 – In my opinion, a stronger link between PFC (specifically) and caffeine ingestion would better support the hypothesis rather than a generic increased activity and excitability neuronal activity in CNS (some experimental data linking PFC activity and caffeine ingestion).

Reply: Thank you very much (we enjoyed writing this paper). We included a neuroimaging study, accordingly.

Merola A, Germuska MA, Warnert EA, Richmond L, Helme D, Khot S, Murphy K, Rogers PJ, Hall JE, Wise RG. Mapping the pharmacological modulation of brain oxygen metabolism: The effects of caffeine on absolute CMRO2 measured using dual calibrated fMRI. Neuroimage. 2017 Jul 15;155:331-343. doi: 10.1016/j.neuroimage.2017.03.028. Epub 2017 Mar 18.

Lines 205 – 207. The Azevedo et. al study actually included some psychological variables that were influenced by the caffeine ingestion.

Reply: Yes, you are right. Study by Azevedo et al. (2016) reported POMS subscales such vigor and fatigue (pre-to-post treatment) and RPE (during the trial). In the present study, we presented a more comprehensive description of psychological responses to treatment (i.e. pre to post RVP test) as well as to exercise.

Line 220 – The hypothesis would be clearer with a direction. “changing psychological responses” is generic and vague.

Reply: ok, changed accordingly

Method

In general, the methods section is long, but I understand that this is due to the high complexity of what was done. In my opinion, a schematic figure summarizing the study design would facilitate its understanding, especially regarding the comparisons that were made. I have some major doubts about the methodological choices, which in my opinion must be clarified in the methods description.

Reply: Thanks for your comment, we agree with you. As told earlier, we designed a comprehensive study to provide TT20km reliability measures (trial 1 vs trial 2; preliminary trial vs baseline trial) and a proof-of-concept of mental fatigue effects (trial 2 vs trial 3; baseline trial vs MF trial) before investigating CAF effects on mentally fatigued individuals. We have no knowledge of supplementation studies presenting a control like this. We have included an illustration of the study design.

Line 243 – How the participant's characteristics were obtained? (vo2 max, Wmax, etc), as no visit for this specific data acquisition was performed? This important information was not mentioned.

Reply: We have informed that eligible cyclists performed a maximal graded test (25 $W \cdot min^{-1}$ increments until voluntary exhaustion) in order to obtain VO_{2MAX} and peak power output (W_{PEAK}). Sorry!

Lines 251-257 – I think it is important to report that even high habitual caffeine intake does not negate the benefits of acute caffeine supplementation, as reported by Gonçalves et. al 2017 (already cited in the present manuscript).

Reply: Ok, inserted accordingly.

Lines 303-305- Why use delta values instead of using the analysis of whole pooled data? I think this option should be explained.

Reply: As answered to your first concern, we designed a study to provide strong, well-controlled results. Thus, we performed the three first sessions in sequential order, as we aimed to provide TT20km reliability data, a proof-of-concept of mental fatigue effects, before investigating CAF effects on mentally fatigued cyclists. Consequently, the study consisted of 3 sequential TT20km (i.e. preliminary, baseline, mental fatigue trials) before CAF and PLA trials (performed in counterbalanced order), so that we were allowed to provide reliability (preliminary trial vs baseline trial) and proof-of-concept measures (baseline trial vs MF trial). However, given these trials were not all performed in counterbalanced order (after random designation), we did not combine baseline and MF TT20km with CAF and PLA TT20km, as this would fail to meet methodological assumptions of multiple comparisons (such as balancing and ordering). In addition, to mitigate inter-individual data variability we expressed MF+CAF and MF+PLA performance as delta alterations from MF TT20km, as reported elsewhere [10]. We have reworded it throughout the manuscript, please, let me know if it is ok.

Line 306- Regarding the performance, why the baseline value used to compare was the MF-baseline and not the Baseline without MF? Caffeine ingestion was used to compare with an impaired performance and not to the best performance of participants. If caffeine+mf could improve the performance similar to a non-mf situation would be a more ecological result rather than this comparison with impaired performance. The author's choice to make this comparison with MF-Baseline needs a further and deeper explanation, as to verify if caffeine ingestion counteracts MF is one of the objectives of this manuscript.

Reply: Sorry! we think our writing approach should have been better, thus allowing a clear interpretation of the results. Please, see amendments throughout the methods section.

Study design – Could athletes be less motivated compared to a baseline? Since the performance comparisons were made with this MF-Baseline situation, why the psychometric measures were not also compared with the baseline? This also should be pointed out in the discussion. (athletes presented reduced motivation in both conditions CAF and PLA).

Reply: We do not think so, all procedures were equally performed in the different sessions so that there is no reason to believe that motivation was different. Additionally, cyclists were informed the study consisted of 5 visits containing the same cycling trial, thus they were not oriented to familiarize themselves with procedures (which could be a factor inducing different motivation). Please, see amendments throughout the methods section explaining our methodological choices.

If my understanding is correct, the baseline used to compare the EEG data was the situation without MF. Why was this different to the performance measures, as this variable was used to explain some of the performance results?

Reply: Sorry, the writing approach should have been clearer. Please, see amendments throughout the methods section.

Lines 540-544 – As reported before, I still cannot understand why the comparison was made to the MF-Baseline situation. If the objective was to verify if CAF counteracts MF in cycling performance, the best performance available should be used as a baseline (control situation). This comparison with an already impaired performance does not make sense in an ecological point of view (the manuscript is based on the idea that the acute caffeine ingestion in mentally fatigued athletes should improve the performance and "return" this performance similar to a non-fatigued state).

Reply: Please, see amendments throughout the methods section.

Lines 485-502 - Applying the same logic of the performance data, in my opinion, the psychological data during the TT20km should also be compared with the baseline data (control situation without mf). This isolated comparison between MF + CAF and MF +

PLA limits the interpretation of the results because it is not known if the values would approximate to a situation without MF (in other words, if acute caffeine ingestion would revert the adverse psychological effects of MF).

Reply: Sorry, the writing approach should have been clearer. Please, see amendments throughout the methods section.

Results

Figure 1 – Why was panel A presented with absolute values (min) and panel B with relative values (% of w)?

Reply: This figure depicts the percentage of alteration in time (panel A) and power output (panel B) induced by MF.

Figure 6 – It appears that the description of the results is different from captions and panels.

Reply: Thank you, we corrected it.

Discussion

Lines 871-877- In my opinion, this discussion about placebo effects in endurance performance could be further explored. This could be the main reason for the no difference in PFC activation between situations and was left undiscussed.

Reply: Thank you. Actually, placebo effects have challenged exercise and sport sciences, mainly the sports nutrition area. We have tried to balance our discussion in view of our dataset (results), considering we have results derived from different controls (reliability and proof of concept) and manipulation (mental fatigue and caffeine supplementation). After some debate among authors and several drafts, we concluded that expanding placebo effects discussion further would unbalance the manuscript (as the study of placebo effects was not an aim of the study). We hope you are happy with our argument.

Line 889-892 – In my opinion, this sentence needs a further explanation. How may the cyclists have been benefited from an alleviated MF fatigue-induced negative sensation? Which variables could be pointed? Is there any experimental study showing this?

Reply: Study by Azevedo et al., [10] showed that caffeine reduced RPE and increase vigor sensation in cyclists under mental fatigue. Moreover, we observed in our study that

mental fatigue sensation increased at a lower level after RVIP test and affect and emotional arousal increased in MF+CAF trial. Overall, these results suggest alleviated mental fatigue-induced negative sensations after CAF ingestion.

Lines 900-902– In my opinion, based on how data was analyzed, this statement could not be true. If only MF situations were compared (MF-baseline x MFCAF and MFPLA), acute caffeine ingestion can be related to a better performance when compared to PLA, but when compared to a non-MF condition (the best performance available) it was not demonstrated. My suggestion is to include the baseline values (without the presence of MF) in this analysis. Once again, in my opinion, it is possible to confirm that caffeine ingestion counteracts the negative effect of MF only if there is a comparison with a "control" condition (without mf). If the MF+CAF was similar to the "control" condition, this statement is true. If "control" condition is better than MF+CAF, the caffeine ingestion was not able to "return" the performance to baseline values.

Reply: In strict terms you are right, we cannot assume that caffeine reverted mental fatigue-reduced performance, instead we can assume that caffeine improves TT20km performance in mentally fatigued cyclists. We have reworded it throughout the manuscript, thus given more accuracy to our assumptions.

Line 797 - Looking at figure 6, can we actually say that psychological states were different between conditions?

Reply: Firstly, we fixed figure 6 as the signals were in contrary direction. Yes, inferential stats has shown CAF effect on them.

As no difference between conditions was identified in absolute RPE, why this difference was demonstrated in rpe/w? In other words, what is the difference between these two measures in terms of psychophysiology measures?

Reply: Thanks for your question. Absolute RPE value is an intrinsic measure of how effortful the exercise was. RPE has been suggested as a template formed from previous experiences, derived from the momentary RPE in relation to the endpoint, useful to athletes to set a pace during exercise and avoid premature fatigue. Thus, in real world scenarios athletes use to set their pace based on their perceived exertion (i.e. RPE) rather than on their velocity/power output. Hence, saying that CAF increased the RPE/w means

that cyclists were able to generate higher power output (i.e. performance) for the same RPE template. Similar results have been reported with different manipulation [11–13].

Pinheiro FA, Santos TM, Pires FO. Conscious distance monitoring and perceived exertion in light-deprived cycling time trial. Physiol Behav. 2016 Oct 15;165:211-6. doi: 10.1016/j.physbeh.2016.07.020. Epub 2016 Jul 29.

Parry D, Micklewright D. Optic flow influences perceived exertion and distance estimation but not running pace. Med Sci Sports Exerc. 2014 Aug;46(8):1658-65. doi: 10.1249/MSS.0000000000000257.

Akers A, Barton J, Cossey R, Gainsford P, Griffin M, Micklewright D. Visual color perception in green exercise: positive effects on mood and perceived exertion.Environ Sci Technol. 2012 Aug 21;46(16):8661-6. doi: 10.1021/es301685g. Epub 2012 Aug 10.

Line 926-930 - How could the CAF ingestion prevent this "cognitive depletion"? The reference indicated to sustain this assumption did not measure this variable. I believe some papers regarding ego depletion could help to discuss this result.

Reply: Thanks for drawn attention to this mistake, the reference was quoted inadequately.

Conclusion

Lines 1005-1007 – As explained before, I disagree with this statement. In my opinion, to confirm that CAF could revert MF effects on performance, a comparison with a non-mf state should be provided.

Reply: Thanks for your comment. We reworded crucial points in the manuscript regarding our assumption, as we really cannot assume that caffeine reverted mental fatigue-reduced performance; instead we can only assume that caffeine improved TT20km performance in mentally fatigued cyclists.

Lines 1012-1014 – I think the data available in this manuscript is not sufficient to support this statement, that is quite broad when it comes to "cerebral responses". I believe a more specific conclusion about the role of PFC activation is necessary.

Reply: We rephrased it, please let us know if you are happy.

References

Reply: thanks, references were checked and fixed.

There are a few minor details related to the references formatting that need to be fixed (i.e. “,” after last author name, etc.)

Reply: thanks, we corrected.

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Caffeine Improved Cycling Trial Performance in Mentally Fatigued Cyclists, Despite Unchanged Prefrontal Cortex Activation

Paulo Estevão Franco-Alvarenga[1], Cayque Brietzke[1], Raul Canestri[1], Márcio Fagundes Goethel[1], Florentina Hettinga[2] , Tony Meireles Santos[3], and Flávio Oliveira Pires[1,4*]

[1]Exercise Psychophysiology Research Group, School of Arts, Sciences and Humanities – University of São Paulo, São Paulo, Brazil

[2]School of Sport, Rehabilitation & Exercise Science - University of Essex, Colchester, United Kingdom.

[3]Federal University of Pernambuco, Recife, Brazil.

[4] Human Movement Science and Rehabilitation Program, Federal University of São Paulo, Santos, São Paulo, Brazil.

* Correspondence:

Flavio O Pires

School of Arts, Science, and Humanities, University of São Paulo, Brazil

Arlindo Bettio Avenue, 1000, 03828-000. Tel: 55+11+3091-8157

e-mail: piresfo@usp.br

ABSTRACT

Purpose: To verify whether caffeine (CAF) could increase the prefrontal cortex (PFC) activation and improve 20 km cycling time trial (TT_{20km}) performance in mentally fatigued cyclists. **Methods:** After preliminary TT_{20km}, twelve recreational cyclists (VO_{2MAX} of 58.9 ± 6.2 $mL \cdot kg \cdot min^{-1}$) performed a familiarization with a cognitive test to induce mental fatigue (MF) and psychological scales. Thereafter, they performed: 2) a baseline TT_{20km}; 3) a mentally fatigued TT_{20km} (MF); 4 and 5) a mentally fatigued TT_{20km} after CAF (MF+CAF) or placebo (MF+PLA) ingestion, in a double-blind, counterbalanced design. Performance and psychological responses were obtained throughout the TT_{20km}, while PFC electroencephalography (EEG) theta wave was obtained before and after the mental fatigue test. **Results:** The mental fatigue-induced increase in EEG theta wave (↑ ~ 4.8 %) was reverted with CAF (↓ 8.8 %) and PLA ingestion (↓ 4.8 %). CAF improved TT_{20km} performance in mentally fatigued cyclists by reducing time ($p = 0.00$; ↓ ~1.7 %) and increasing W_{MEAN} ($p = 0.00$; ↑ ~3.6%), when compared to MF+PLA. The RPE-power output ratio was lower ($p = 0.01$), but affect ($p = 0.018$), motivation ($p = 0.033$) and emotional arousal ($p = 0.001$) were greater throughout the TT_{20km} in MF+CAF than in MF+PLA. **Conclusions:** CAF ingestion improved TT_{20km} performance and psychological responses in mentally fatigued cyclists, despite the unaltered PFC activation.

Keywords: Fatigue; Placebo; RPE; Pacing; Supplementation

INTRODUCTION

A body of literature has shown that mental fatigue impairs endurance cycling performance [1–3]. Recently, a study by Pires et al., [3] also found that mental fatigue reduced 20 km cycling time trial (TT_{20km}) performance by ~2.7 % and ~6.5 % when results were expressed as time and mean power output (W_{MEAN}), respectively. The authors related such an impaired cycling performance to a decreased prefrontal cortex (PFC) activation [4,5], as interpreted by the increased electroencephalography (EEG) theta wave at the 10th and 20th km of the trial. Importantly, this altered PFC activation during the TT_{20km} may have resulted from the high-demanding cognitive task performed prior to the cycling trial, as an increased PFC EEG theta wave was readily observed during the cognitive test [3]. Although the underlying mechanisms were not fully clarified, mental fatigue may have affected the higher-order cognitive control, thereby reducing the ability to deal with attentional control, encoding and storage of relevant information [6], leading to a greater perceived cost-future reward relationship and aversive sensations during exercise [2,7]. In this regard, as the exercise disengagement/investment is related to the mental representation of the sense of effort [8], manipulations capable of unbalancing the incentive-performance and effort-performance relationship (such as mental fatigue) may affect performance during exercise [2,9]. Therefore, recreational mentally fatigued cyclists may have less cognitive ability to use inhibitory control and deal with aversive sensations when regulating their pace during exercise.

Cycling trials in which a specific distance has to be covered as fast as possible are a realistic scenario that resembles conditions met in cycling training and competitions routines [3,10]. In this scenario, cyclists may base their pace on psychological responses such as the ratings of perceived exertion (RPE), as it has been suggested that athletes avoid premature fatigue and maximize performance by using a RPE template formed from previous experiences, derived from the momentary RPE in relation to the endpoint [11,12]. Consequently, mental fatigue may be a threat for a successful cycling pacing and performance regulation, as mentally fatigued cyclists may perceive a higher than expected RPE for the same power output during the trial [13]. They may have insufficient

motivation to overcome exercise-derived aversive sensations, thereby impairing performance [3,14]. Hence, interventions capable to counteract negative mental fatigue effects on endurance performance may be helpful to improve performance, particularly in recreational athletes, as they may regularly experience mental fatigue due to their high-load aerobic training routines combined with a strict-life style that encompasses dietary restrictions, longer work-days, reduced time for recovery and restricted social life [2,3].

Mental fatigue is likely associated with an elevated ATP hydrolysis and increased adenosine concentrations in the central nervous system (CNS) [15,16]. This condition has been associated with an inhibited release of excitatory neurotransmitters (such as dopamine) which reduces arousal, spontaneous behavior and affect (i.e. pleasure) during exercise [16–18]. In this regard, a substance with the potential to counteract mental fatigue effects is caffeine (CAF), as it has been suggested that CAF increases neuronal activity and excitability of the CNS by blocking neuronal A_1 adenosine receptors [17,19]. For example, a neuroimaging study observed that CAF improved the tissue oxygen extraction and reduced the cerebral metabolic rate of oxygen consumption [20]. Consequently, one may hypothesize that CAF counteracts the mental fatigue-altered PFC activation, thereby improving cycling performance in mentally fatigued cyclists. In fact, a recent study [1] provided insightful results as mentally fatigued individuals increased their cycling time-to-exhaustion after CAF ingestion (when compared to a mental fatigue trial without CAF). However, neither PFC EEG measures during the high-demanding cognitive task nor psychological measures such as motivation, affect and emotional arousal during exercise were included, therefore inferences to a more realistic distance-based time trial scenario are still required [3].

The present study verified whether CAF improved PFC activation and TT_{20km} performance in mentally fatigued cyclists. We also verified if CAF ingestion altered psychological responses to a TT_{20km} in mentally fatigued cyclists. We hypothesized that CAF ingestion would attenuate the mental fatigue-reduced PFC activation [3], improving TT_{20km} performance of mentally fatigued cyclists.

Moreover, we expected that CAF would reduce RPE, and increase affect, motivation and emotional arousal during exercise.

MATERIALS AND METHODS

Participants

The sample size was calculated through an equation suggested elsewhere ($n = 8e^2/d^2$; n, e, and d denote the required sample size, coefficient of variation and magnitude of the treatment, respectively) [21], assuming e of 1.1 % for TT_{20km} performed by recreational cyclists [22] and a conservative d of 1.0 %, thus resulting in ~ 10 participants. However, considering a possible sample loss of ~20 %, 12 non-professional trained cyclists (means and SDs of 34.3 ± 6.2 years; 179.3 ± 5.1 cm; 77.6 ± 6.8 kg) classified as performance level 3 (means and SDs of VO_{2MAX} = 58.9 ± 6.2 $mL \cdot kg \cdot min^{-1}$; W_{PEAK} = 367.0 ± 32.5 W) according to criteria suggested elsewhere [23] volunteered to take part in this study. They had a training frequency of 4.7 ± 2.3 sessions/week (283.7 ± 138.6 km/week) and a training experience of ~ 6.5 years (competing at regional and national tournaments) when the study was conducted. They were non-smokers and free from cardiovascular, visual, auditory and cognitive disorders. Three of them were non-consumers (≤ 40 mg of CAF per day), five were occasional consumers (≤ 250 mg of CAF per day) and four were daily consumers (250 ≤ consumption ≤ 572 mg of CAF per day), according to a proposed classification [24]. Importantly, CAF has been suggested as an ergogenic aid capable of improving endurance performance, regardless of habitual caffeine consumption [25,26].They were oriented to avoid consumption of stimulant (coffee, energy drink etc.) and alcoholic beverages, as well as intense exercise for the 48 h preceding the sessions. Experimental procedures, risks, and benefits were explained before collecting their written consent form signature. The procedures were previously approved by a local Ethics Committee (Process: 63787816.1.0000.5390) and performed according to the Declaration of Helsinki.

Study Design

The design of the present study encompassed 5 sessions, as depicted in Figure 1. Firstly, cyclists performed a TT_{20km} during a preliminary session (visit 1) and those who completed the trial within 33 min, were eligible to participate in the study. This criterion was based on previous TT_{20km} studies and adopted to homogenize the sample and reduce the data variability [3,27–29]. Afterwards, eligible cyclists were familiarized with a short version (~ 5 min) of the rapid visual information processing (RVIP) test and psychological scales. Thus, after preliminary session eligible cyclists attended to more four sessions: 2) Baseline: cyclists performed a baseline TT_{20km}; 3) Mental Fatigue (MF): cyclists completed a TT_{20km} after performing a 40 min RVIP test; 4 and 5) Experimental trials: cyclists completed a TT_{20km} after ingesting either CAF (MF+CAF) or placebo (MF+PLA) before the 40 min RVIP test.

Briefly, we designed the three first sessions to provide a strong, adequate control before the investigation of CAF effects on mentally fatigued cyclists. Thus, sessions 1 (preliminary TT_{20km}) and 2 (baseline TT_{20km}) were designed to provide TT_{20km} performance measures reliability. Moreover, despite most studies have consistently shown that mental fatigue impairs endurance performance [13], only one showed mental fatigue effects on TT_{20km} [3]. Hence, rather than assuming it, we confirmed that mental fatigue impaired TT_{20km} performance (a proof-of-concept) by comparing session 2 vs session 3 (baseline TT_{20km} vs MF TT_{20km}). Consequently, sessions 4 and 5 were designed to investigate if CAF may improve TT_{20km} performance in mentally fatigued cyclists, so that MF+CAF and MF+PLA trials were performed in a double-blinded, counterbalanced order. The study was finished within 30 days, the sessions were interspersed by a 3-7 days washout period, performed at the same time of the day, under controlled temperature (~24◦C) and humidity (50–60%). Psychological responses such as RPE, motivation, emotional arousal and affect were measured every 2 km through the TT_{20km}, while EEG, motivation, emotional arousal, and mental fatigue sensation were also obtained before and after the RVIP test. After the study conclusion, cyclists performed a maximal graded test (25 $W \cdot min^{-1}$ increments until voluntary exhaustion) in order to obtain their VO_{2MAX} and peak power output (W_{PEAK}).

*** Figure 1 ***

Mental Fatigue Protocol

The RVIP test was performed in a silent and illuminated room [30]. Cyclists sat comfortably on a chair, frontally to a 17 inches colored monitor, and wore an earphone damper to avoid noise distractions. The RVIP test consisted of a 40 min high-demanding cognitive task, which randomly displayed single numbers (numbers from 1 to 9 being displayed isolated) in a white box in the center of the monitor, in a rate of 100 numbers per minute (one number per 600 milliseconds). They were asked to press the space bar of a standard keyboard every time they identified a sequence of three even (e.g., 2, 4, 6; 4, 6, 8 etc.) or odd numbers (e.g., 3, 5, 7; 3, 9, 7 etc.), shown eight times a minute. Cognitive performance was measured as false alarms (expressed as arbitrary units; a.u.), reaction time (s) and percentage of accuracy answers (i.e. correct numerical sequences %).

Caffeine and Placebo Ingestion

We followed the recommendations of the International Society of Sports Nutrition (ISSN) position for CAF ingestion [24]. Briefly, it has been suggested that 3 to 6 $mg \cdot kg^{-1}$ of body mass of CAF significantly improve endurance performance in trained athletes approximately 1 h post-ingestion [24]. In order to accomplish this recommendation, participants ingested 5 $mg \cdot kg^{-1}$ of body mass of CAF or PLA immediately before the RVIP test (~ 50 min before the cycling TT_{20km} commencement). The CAF and PLA capsules were formulated to have the same appearance (i.e. form, size and color) and contain the same taste and smell, thereby ensuring that cyclists could not identify differences between them. CAF was manipulated as previously reported in a mental fatigue-caffeine study [1]. In contrast, PLA was manipulated in cellulose capsules containing inert substances such as a lubricant, magnesium stearate, and magnesium silicate. Importantly, neither participants nor researchers appointed to the data collection were aware of the intervention. Likewise, researchers

appointed to data analysis were blinded to manipulations, thus characterizing the present study as a truly double-blind study.

Cycling Time Trial (TT_{20km})

Cyclists performed the baseline, MF, MF+CAF and MF+PLA TT_{20km} having only distance as available feedback, that is they were blinded to feedback such as time, cadence, speed, power output, and heart rate. Cyclists used a road bicycle (Giant®, New York, USA) attached to a cycle simulator (Computrainer, Racer Mate ® 8000, Seattle, USA) that provided power output (W), cadence (rpm) and speed ($km \cdot h^{-1}$) data throughout the trials. The device was calibrated before each test according to the manufacturer's instructions. The bike was individually adjusted according to cyclists' preferences and they were allowed to drink water *ad libitum* during the trials. The time to complete the TT_{20km} and the W_{MEAN} recorded throughout the trial were used as performance measures. Furthermore, power output data were averaged every 2 km to analyze pacing strategy.

Measures and Instruments

Electroencephalography (EEG)

Previous EEG studies have suggested that EEG theta wave is a slow frequency EEG band sensitive to distinguish a mental fatigue state [3–5]. Additionally, theta rather than alpha wave may be a reliable distinguisher of changes in cognitive processing as mental fatigue progresses, as frontal cortex EEG theta wave is correlated with the percentage of accuracy answers (i.e. error rate) during high-demanding cognitive tasks [5]. Hence, PFC activation was continuously obtained through an EEG unit (Emsa®, EEG BNT 36, TiEEG, Brazil) at the Fp1 position, according to the international EEG 10-20 system [31]. This position was ensured according to frontal and sagittal planes, referenced to mastoid. The EEG was recorded at a 600 Hz sampling frequency, through active electrodes (Ag-AgCl) with resistance ~5 KΩ. After exfoliation and cleaning, electrodes were fixed with a conductive gel, adhesive tape, and medical strips. The EEG signal was recorded during 3 min rest, immediately

before and after the RVIP test, when participants were completely calm, maintaining their eyes closed and avoiding head and trunk movements.

The EEG signal was amplified (gain of $1 \cdot 10^3$) and filtered with a digital notch (60 Hz), thereafter a 1-30 Hz bandpass filter was applied. EEG signal showing spectral leakage, defined as a signal $\geq$ 100 μv, were considered as artifacts (n = 1-2, depending on the moment of the experimental setup) and were excluded from the analysis [31]. Furthermore, data recorded during the first and last 30 s of a 180 s time window were removed to avoid eventual noise associated with the participants' movements when expecting the EEG record start and stop. Thereafter, EEG data were analyzed in frequency domains through a fast-Fourier transformation so that the total power spectral density (tPSD) of the theta wave (3 – 7 Hz) was calculated over the most steady (i.e. lowest SD) 30 s window (determined through an algorithm implemented in Matlab® environment).

Psychological Responses

Responses of mental fatigue sensation, emotional arousal and motivation were obtained before and immediately after the RVIP test. Briefly, the mental fatigue sensation was rated through a 100 mm visual analogue scale (VAS), then cyclists were required to answer "How mentally fatigued you feel now?" having 0 (zero) as "none at all" and 100 as "maximal" mental fatigue, as reported elsewhere [32]. The emotional arousal was assessed through a 6-points felt arousal scale (FAS) that ranks the emotional arousal within categories ranging from "low activation" to "high activation" [33]. The perception of high emotional arousal may be interpreted as a state of "worked-up" whilst perception of low emotional arousal, as a state of "relaxation". Moreover, motivation was assessed through a 10 points Likert scale having two opposite motivational descriptors, that is 0 (zero) as "not all motivated" and 10 as "extremely motivated" [34,35]. These responses, expressed as arbitrary units (a.u.), were compared between pre and post RVIP test.

Furthermore, emotional arousal, motivation, affect and RPE responses were obtained every 2 km of the TT_{20km}. Affect responses (pleasure/displeasure) were obtained by using the 11-points

feeling scale (FS), as suggested elsewhere [36]. This single-item bipolar scale (-5 to +5) uses descriptors as "neutral" (zero), "very good" (+5) and "very bad" (-5) to rate the affective valence. Furthermore, RPE was obtained through a 15-points Borg scale, as suggested elsewhere [37]. In order to verify possible mental fatigue-induced psychological alterations during exercise, motivation, FAS, FS and RPE (expressed as a.u.) were analyzed every 2 km. Given the comparable absolute RPE responses in control and mental fatigue, although the reduced power output values under mental fatigue [3], we also calculated the RPE-power output ratio (RPE_W) for every 2 km of the TT_{20km}.

Statistical Analysis

Gaussian distribution and homoscedasticity were previously checked through Shapiro-Wilk and Levene tests, respectively, and results were reported as mean and standard deviation (± SD).

Firstly, we checked the reliability on performance measures by comparing preliminary TT_{20km} (session 1) and baseline TT_{20km} (session 2), and reporting the typical error of measurement (expressed as a variation of the grand mean) and the correlation coefficient between them [21].

Secondly, as a proof-of-concept of mental fatigue effects we verified if performance in MF TT_{20km} was impaired when compared to baseline TT_{20km}. Therefore, time and W_{MEAN} responses in baseline and FM TT_{20km} were compared through a paired T-student test (session 2 vs session 3). Particularly in MF session (session 3), we also compared pre to post RVIP test alterations in EEG theta power, VAS, FAS and motivation through a paired T-student test.

Effects of CAF ingestion on mentally fatigued cyclists were assessed in different ways. Firstly, to mitigate the impact of inter-individual variability (between sessions) we expressed EEG and psychological responses (i.e. sensation of fatigue, emotional arousal, and motivation) as Δ values from pre-treatment (pre to post RVIP measures) and compared MF+CAF and MF+PLA responses through a paired T-student test. Secondly, we compared cognitive performance (i.e. false alarms, reaction time and accuracy of answers averaged during the RVIP test) between MF+CAF and MF+PLA sessions through a paired T-student test.

Furthermore, we confirmed if CAF improved TT_{20km} performance (W_{MEAN} and time) in mentally fatigued cyclists. Accordingly, to mitigate the impact of inter-individual variability we expressed TT_{20km} performance as Δ values from MF TT_{20km}, and compared MF+CAF and MF+PLA through a paired T-student test. In addition, we analyzed pacing (i.e. power output) and psychological responses (i.e., RPE, RPE_W, motivation, FAS and FS) during the MF+CAF and MF+PLA through a 10 x 2 mixed model having distance (2nd, 4th up to 20th km) and condition (MF+CAF *vs* MF+PLA) as fixed factors, and cyclists as the random one. The AIC index (Akaike's information criterion) determined the covariance matrix that best fitted to the dataset (Compound Symmetric, First-order Autoregressive homogeneous and heterogeneous, First-order Autoregressive Moving Average, and Toeplitz homogeneous and heterogeneous), and the Bonferroni test corrected p values in multiple comparisons.

We reported the post-hoc ES analysis (expressed as *d*-Cohen) to make eventual comparisons with previous studies possible [3,28], so that ES was interpreted as small (< 0.2), moderate (0.2 to 0.6), large (0.6 to 1.2), very large (1.2 to 2.0), and extremely large (≥ 2.0), as suggested elsewhere [38]. Results were significant when $p \leq 0.05$.

RESULTS

As part of the study control, we checked the reliability of performance measures. There was no difference in time ($p = 0.81$; $d = 0.074$, small ES) and W_{MEAN} ($p = 0.27$; $d = 0.066$, small ES) between preliminary (32.8 ± 1.3 min and 262.3 ± 37.5 W) and baseline (32.7 ± 1.4 min and 260.0 ± 32.0 W) sessions. The typical error of measurement and correlation between preliminary (trial 1) and baseline (trial 2) sessions were 0.8 % and $r = 0.94$, and 3.1 % and $r = 0.96$ for time to complete the trial and W_{MEAN}, respectively.

Proof-of-Concept of Mental Fatigue Effects

As a proof-of-concept, we verified if TT_{20km} performance was impaired by mental fatigue, given the 0.9 ± 0.7 % increase in time to complete the trial (32.7 ± 1.4 min vs 33.0 ± 1.4 min; p = 0.00; *d* = 2.41, extremely large ES) and the 2.2 ± 1.6 % reduction in W_{MEAN} (260 ± 32 W vs 254.3 ± 29.7 W; p = 0.00; *d* = 2.87, extremely large ES) in mental fatigue trial when compared to baseline. Figure 2 depicts the percentage of alteration in time (panel A) and power output (panel B) from baseline TT_{20km}.

Furthermore, we observed that the RVIP test changed PFC activation in MF session, as we observed a ~ 4.8 ± 7.1 % increase in EEG theta band from pre to post RVIP test (p = 0.03; *d* = 1.53, very large ES). Accordingly, cyclists rated increased mental fatigue sensation (35.0 ± 16.9 vs 73.3 ± 12.1 a.u.; p = 0.000; *d* = 3.40, extremely large ES), reduced motivation (7.6 ± 1.9 vs 6.0 ± 2.9 a.u.; p = 0.009; *d* = 3.17, extremely large ES) and lower emotional arousal (4.7 ± 1.4 vs 3.8 ± 1.5 a.u.; p = 0.002 *d* = 2.73, extremely large ES) when comparing pre to post RVIP test responses. Mentally fatigued cyclists showed a reaction time of 37.0 ± 12.4 s, false alarms of 22.4 ± 17.4 and accuracy of 41.8 ± 16.1% during the RVIP test.

*** Figure 2 ***

Caffeine Effects on EEG, Psychological and Cognitive Performance Responses in Mentally Fatigued Cyclists

In contrast to the ~ 4.8 ± 7.1 % increase in EEG theta wave found in MF condition, we observed a ~ 8.8 ± 13.9 % and ~ 4.8 ± 17.9 % reduction in EEG theta wave from pre to post RVIP test in MF+CAF and MF+PLA sessions, respectively (Figure 2). Importantly, the Δ alteration in PFC activation was comparable between MF+CAF and MF+PLA sessions (p = 0.25; *d* = 0.50, moderate ES).

Regarding the RVIP test-induced psychological alterations, mental fatigue sensation increased from pre to post RVIP test in both MF+CAF (↑ 65.7 ± 105.4%) and MF+PLA sessions (↑ 114.8 ± 113.0%), but the Δ alteration was significantly higher in MF+PLA than in MF+CAF (p = 0.02; *d* = 0.70, large ES). In contrast, there was an increase in emotional arousal in MF+CAF (↑ 11.4 ± 15.8%) but a decrease in MF+PLA (↓ 18.1 ± 24.2%), thus Δ alteration from pre to post RVIP test was significantly different between conditions (p = 0.01; *d* = 1.51, very large ES). Furthermore, motivation changed slightly from pre to post RVIP test in MF+CAF (↓ 4.6 ± 15.5%) and MF+PLA (↑ 3.3 ± 31.7%), therefore no significant Δ alterations were observed between conditions (p = 0.67; *d* = 0.15, small ES).

Comparable cognitive performance was observed between MF+CAF and MF+PLA, as Δ alterations of reaction time (38.0 ± 14.5 s *vs* 39.8 ± 13.8 s, respectively; p = 0.39; *d* = 0.13, small ES), false alarms (19.7 ± 18.1 *vs* 13.4 ± 11.2, respectively; p = 0.23; *d* = 0.42, moderate ES) and accuracy (46.4 ± 16.1% *vs* 46.8 ± 17.3%, respectively; p = 0.83; *d* = 0.024, small ES) were not significantly different between conditions.

Caffeine Effects on TT_{20km} Performance and Pacing in Mentally Fatigued Cyclists

Mentally fatigued cyclists significantly improved TT_{20km} performance in CAF when compared to PLA ingestion. The 1.8 ± 1.4 % improvement in time to complete the trial with CAF ingestion (32.4 ± 1.2 min) was significantly greater (p = 0.002, *d* = 2.36, extremely large) than the 0.09 ± 1.5 % improvement with PLA ingestion (33.0 ± 1.2 min). Accordingly, the 4.8 ± 4.1 % improvement in W_{MEAN} in MF+CAF (265.8 ± 28.2 W) was significantly greater (p = 0.001, *d* = 2.72, extremely large ES) than the 0.7 ± 3.9 % improvement in MF+PLA session (256.0 ± 25.3 W).

Cyclists adopted a similar "J-shaped" pacing profile throughout the MF+CAF and MF+PLA trials. Multiple comparisons revealed a condition (*F* = 11.62, p = 0.005, *d*= 1.45, very large ES) and a distance main effect (*F* = 17.49, p = 0.000, *d* = 1.78, very large ES) in power output, despite no condition by distance interaction effect was observed (*F* = 0.28, p = 0.97, *d* = 0.23, small ES). Figure

3 (panels A and B) showed performance Δ values from MF TT_{20km} while figure 4 depicted pacing responses.

Figure 3

***Figure 4 ***

Caffeine Effects on TT_{20km} Psychological Responses in Mentally Fatigued Cyclists

Comparable results were observed in absolute RPE values, as neither a condition main effect (F = 2.24; p = 0.16; d = 0.63, very large ES) nor a condition by distance interaction effect (F = 1.18; p = 0.33; d = 0.46, large ES) was detected, despite the distance main effect in absolute RPE values (F = 12.27, p = 0.000, d = 1.43 extremely large ES). However, there was a significant condition main effect (F = 10.32; p = 0.005, d = 1.37, extremely large ES) as well as a distance main effect (F = 4.28, p = 0.001, d = 0.82, large ES) in RPE_W data, as the increase in RPE_W during the TT_{20km} was lower in CAF than in PLA. However, no condition by distance interaction effect was found in RPE_W (F = 1.29, p = 0.278, d = 0.48, large ES). Overall RPE responses were shown in Figure 5 (panel A and B).

***Figure 5 ***

Regarding the remaining psychological responses, a condition main effect (F = 5.72, p = 0.018, d = 1.02 large ES) and a distance by condition interaction effect (F = 2.29, p = 0.019, d = 0.65 large ES) was found in affect, as cyclists reported higher affect in MF+CAF than in MF+PLA when they were spurting at the end of the trial (p = 0.000). However, no distance main effect was detected in affective valence (F = 1.47, p = 0.169, d = 0.52 moderate ES). In contrast, neither a distance main effect (F = 0.45, p = 0.90, d = 0.29 moderate ES) nor a distance by condition interaction effect (F = 0.87, p = 0.55, d = 0.40 moderate ES) was observed in motivation. Nevertheless, a condition main effect (F = 4.61, p = 0.033, d = 0.92, large ES) was observed so that motivation was greater in mentally

fatigued cyclists after in MF+CAF trial. Accordingly, although neither a distance main effect (F = 0.78, p = 0.64, d = 0.38 moderate ES) nor a distance by condition interaction effect (F = 0.88, p = 0.54, d = 0.40 moderate ES) was found in emotional arousal. However, mentally fatigued cyclists rated higher arousal throughout the TT_{20km} in CAF than PLA (F = 11.03, p = 0.001, d = 1.42 very large ES).

***Figure 6 ***

DISCUSSION

The present study was designed to investigate if CAF ingestion may revert mental fatigue effects on PFC activation, thus improving cycling time trial performance in mentally fatigued recreational cyclists. Results showed that CAF reverted the mental fatigue-reduced TT_{20km} performance, despite the comparable CAF and PLA effects on PFC activation. Additionally, CAF reduced RPE and changed other psychological responses throughout the trial. Then, results suggest that CAF is capable to revert the mental fatigue-reduced cycling time trial performance, but challenged its role in cortical activation.

Proof-of-concept of mental fatigue effects on cycling performance

Most studies have shown that mental fatigue impairs endurance performance , but only one showed that mental fatigue impaired TT_{20km} performance [3]. This study suggested that the reduced TT_{20km} performance was possibly related to a mental fatigue-reduced PFC activation. Hence, as a proof-of-concept, firstly we confirmed that mental fatigue affected PFC activation and TT_{20km} performance. We found a change in PFC activation after the RVIP test, indicated by the increased slow-frequency EEG band suggested to distinguish mental fatigue states [3–5]. Moreover, cyclists rated a higher fatigue sensation and lower motivation and emotional arousal after this high-demanding cognitive task. Accordingly, when comparing baseline and MF trials we observed that mental fatigue

reduced cycling performance outcomes after the reliability measures have evidenced that performance was steady (i.e. no learning or training effects from preliminary to baseline trial). Thus, together with others [3], this part of the present study reinforced the notion of a likely connection between PFC activation and impaired TT_{20km} performance. Briefly, it has been proposed that TT_{20km} is a self-paced exercise that requires superior inhibitory control and ability to deal with aversive sensations [2,9], and that PFC is involved in proactive, goal-directed behavior [3,26,39,40]. Therefore, although we have not measured PFC activation during TT_{20km} we found an altered PFC activation readily after the RVIP test, showing that PFC activation may have played a role on TT_{20km} performance in mentally fatigued cyclists [3].

Caffeine effects on high-demanding cognitive task responses

Although the mechanism underlying mental fatigue effects is not fully understood, the reduced PFC activation could be a result of an enhanced cerebral ATP hydrolysis-derived adenosine concentration during cognitive overload [15,16]. Then, we had also hypothesized that CAF ingestion may counteract mental fatigue effects as CAF blocks neuronal A_1 adenosine receptors and improves the neuronal activity and excitability of the CNS [17,19]. However, we observed that both CAF and PLA similarly increased the PFC activation when expressed as pre-to-post RVIP changes. Accordingly, a recent study also reported similar cortical changes to CAF and CAF-perceived PLA ingestion, thereby challenging the effects of CAF ingestion on cortical activation [29]. It has been proposed that the expectation of receiving a given substance (such as CAF) during a PLA ingestion may induce cortical changes in the direction of the active substance [29,41]. Thus, perhaps the cyclists may have experienced some PLA effects as reported elsewhere [29], although we have used a true double-blind design in the present study. Therefore, although knowing that they had 50% chance of ingesting CAF or PLA in each experimental session, the uncertainty about the substance ingested may have led them to expect some CAF effects. However, PLA effects in laboratory settings have been poorly understood and require future studies.

Interestingly, both CAF and PLA also improved cognitive performance responses to the RVIP test. However, CAF attenuated the mental fatigue-induced negative sensations rather than PLA, as indicated by the lower fatigue sensation and higher emotional arousal in CAF than in PLA after the RVIP test. Somehow, the cycling performance in MF+CAF trial may have benefited from an alleviated mental fatigue-induced negative sensations before starting the TT_{20km}.

Caffeine effects on TT_{20km} performance and psychological responses in mentally fatigued cyclists

Actually, regardless of a "J-shaped" pacing strategy adopted in all experimental sessions, CAF ingestion improved TT_{20km} performance expressed as time and W_{MEAN} when compared to PLA. Interestingly, mentally fatigued cyclists showed an "improved psychological state" after CAF ingestion, given the reduced RPE_W ratio, and increased affect, motivation and emotional arousal during TT_{20km}.

It has been proposed that a successful distance-based cycling trial performance such as a TT_{20km}, is related to the cognitive ability to preserve inhibitory control while dealing with aversive sensations [2,9], because cyclists would be required to adequately evaluate the perceived cost-future reward relationship during exercise in order to maximize their pace and complete the trial as fast as possible. Consequently, mental fatigue is considered as a threat to a successful TT_{20km} performance. When compared to PLA, CAF reduced the mental fatigue-negative sensations after the RVIP test. Likewise, mentally fatigued cyclists completed the TT_{20km} reporting lower RPE_W, higher affective valence, motivation and emotional arousal after CAF ingestion. Perhaps, CAF prevented cyclists from the RVIP test-induced cognitive depletion before the trial, thereby allowing them to complete the TT_{20km} under an "improved psychological state" when compared to PLA [2]. Somehow, these improved psychological responses are likely associated with an improved cycling performance as reported elsewhere [1].

Methodological aspects and practical implications

CAF has been suggested as a powerful aid in improving endurance performance regardless of habitual caffeine consumption [25], mainly through its action on the CNS [1,19,42]. It should be pointed out that peripheral effects such as an increased glycolytic flux, mitochondrial oxidation rate and lipid oxidation-induced muscle glycogen sparing [42] have been reported in millimolar doses (supra-physiological) of CAF [19,43]. Therefore, considering that we used oral doses ~ 100 times lower than millimolar dosage [43], it is unlikely that mentally fatigued cyclists have improved cycling performance due to a peripheral CAF action [44].

Recently, a study showed that CAF reverted negative mental fatigue effects on cycling time-to-exhaustion performance [1] while another verified that mental fatigue impaired TT_{20km} performance [3]. Thus, we combined both hypotheses, as cyclists may experience mental fatigue and use supplements as CAF in training and competitions. In fact, a study by Stewart et al., [45] verified that cyclists committed to the sport may perceive pressure to use supplements to improve performance. In contrast, cyclists may experience mental fatigue due to the high-load aerobic training routines combined with a strict-life style in daily activities [2,3]. Thus, our results have practical implications as we verified that CAF counteracted the mental fatigue-reduced performance during a cycling trial that resembles the conditions met in cycling competitions and training sessions [10,27].

In order to potentiate our manipulation we administered CAF readily before the RVIP test. Because the oral CAF ingestion has a ~ 45-60 min time course [24] cyclists had to ingest CAF immediately before the 40 min RVIP test, as the ingestion after the RVIP test could have missed some mental fatigue effects. Despite most effects likely occurring from 45-60 min after CAF ingestion, we cannot ensure that cyclists did not experience some CAF effects during the RVIP. Actually, cyclists reported attenuated psychological changes readily after the RVIP test when they ingested CAF. Future studies may verify if other soluble central-action compounds or tasting CAF (instead of ingesting) may also counteract mental fatigue effects on performance.

CONCLUSIONS

The present study showed that CAF improved TT_{20km} performance in mentally fatigued cyclists, regardless of alterations in PFC activation. Furthermore, CAF ingestion attenuated the mental fatigue-induced negative sensations, thus reducing RPE and increasing affect and emotional arousal during the cycling trial.

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FIGURES' CAPTION

Figure 1. Schematic representation of the study design. TT_{20km} is 20 km cycling time trial. MF is mental fatigue; CAF is caffeine; PLA is placebo; RVIP is rapid visual information processing; EEG is electroencephalography.

Figure 2. Performance changes from baseline to mental fatigue trial expressed as time to complete the TT_{20km} (panel A) and W_{MEAN} (panel B).

Figure 3. Performance changes in mentally fatigued cyclists after caffeine (MF+CAF) and placebo (MF+PLA) ingestion. Data of time to complete the TT_{20km} (panel A) and W_{MEAN} (panel B) were reported as mean ± SD. * indicates that time to complete TT_{20km} ($p = 0.002$) and W_{MEAN} ($p = 0.001$) were significantly different.

Figure 4. Power output responses of mentally fatigued cyclists throughout the TT_{20km} after caffeine (MF+CAF, filled circles) and placebo (MF+PLA, open circles) ingestion. # is condition main effect ($p = 0.005$); * is distance main effect ($p = 0.000$). Data were reported as mean ± SD.

Figure 5. Absolute RPE (panel A) and RPE_W (panel B) values in mentally fatigued cyclists throughout the TT_{20km} after caffeine (MF+CAF, filled circles) and placebo (MF+PLA, open circles) ingestion. # is condition main effect for RPE_W ($p = 0.005$); * is distance main effect for RPE ($p = 0.000$) and RPE_W ($p = 0.001$). Data were reported as mean ± SD.

Figure 6. Psychological responses in mentally fatigued cyclists throughout the TT_{20km} after caffeine (MF+CAF, filled circles) and placebo (MF+PLA, open circles) ingestion. # is a condition main effect in affective valence ($p = 0.018$), motivation ($p = 0.033$) and emotional arousal ($p = 0.001$); Condition by distance interaction effects are shown in boxes. Data were reported as mean ± SD.

Caffeine Improved Cycling Trial Performance in Mentally Fatigued Cyclists, Despite Unchanged Prefrontal Cortex Activation

Bullet Points

1- Mental fatigue (MF) impaired performance in a 20 km cycling time trial (TT_{20km}).

2- The MF-reduced prefrontal cortex activation was probably related to pacing regulation.

3- Caffeine (CAF) increased the TT_{20km} performance in mentally fatigued cyclists.

4- CAF reduced RPE and increased affect and motivation of mentally fatigued cyclists.

5- Effects of CAF on the MF-reduced prefrontal cortex activation remain unclear.

Caffeine Improved Cycling Trial Performance in Mentally Fatigued Cyclists, Despite Unchanged Prefrontal Cortex Activation

Paulo Estevão Franco-Alvarenga[1], Cayque Brietzke[1], Raul Canestri[1], Márcio Fagundes Goethel[1], Florentina Hettinga[2] , Tony Meireles Santos[3], and Flávio Oliveira Pires[1,4*]

[1]Exercise Psychophysiology Research Group, School of Arts, Sciences and Humanities – University of São Paulo, São Paulo, Brazil

[2]School of Sport, Rehabilitation & Exercise Science - University of Essex, Colchester, United Kingdom.

[3]Federal University of Pernambuco, Recife, Brazil.

[4] Human Movement Science and Rehabilitation Program, Federal University of São Paulo, Santos, São Paulo, Brazil.

* Correspondence:

Flavio O Pires

School of Arts, Science, and Humanities, University of São Paulo, Brazil

Arlindo Bettio Avenue, 1000, 03828-000. Tel: 55+11+3091-8157

e-mail: piresfo@usp.br

ABSTRACT

Purpose: To verify whether caffeine (CAF) could increase the prefrontal cortex (PFC) activation and improve 20 km cycling time trial (TT_{20km}) performance in mentally fatigued cyclists. **Methods:** After preliminary TT_{20km}, twelve recreational cyclists (VO_{2MAX} of 58.9 ± 6.2 $mL \cdot kg \cdot min^{-1}$) performed a familiarization with a cognitive test to induce mental fatigue (MF) and psychological scales. Thereafter, they performed: 2) a baseline TT_{20km}; 3) a mentally fatigued TT_{20km} (MF); 4 and 5) a mentally fatigued TT_{20km} after CAF (MF+CAF) or placebo (MF+PLA) ingestion, in a double-blind, counterbalanced design. Performance and psychological responses were obtained throughout the TT_{20km}, while PFC electroencephalography (EEG) theta wave was obtained before and after the mental fatigue test. **Results:** The mental fatigue-induced increase in EEG theta wave (↑ ~ 4.8 %) was reverted with CAF (↓ 8.8 %) and PLA ingestion (↓ 4.8 %). CAF improved TT_{20km} performance in mentally fatigued cyclists by reducing time ($p = 0.00$; ↓ ~1.7 %) and increasing W_{MEAN} ($p = 0.00$; ↑ ~3.6%), when compared to MF+PLA. The RPE-power output ratio was lower ($p = 0.01$), but affect ($p = 0.018$), motivation ($p = 0.033$) and emotional arousal ($p = 0.001$) were greater throughout the TT_{20km} in MF+CAF than in MF+PLA. **Conclusions:** CAF ingestion improved TT_{20km} performance and psychological responses in mentally fatigued cyclists, despite the unaltered PFC activation.

Keywords: Fatigue; Placebo; RPE; Pacing; Supplementation

INTRODUCTION

A body of literature has shown that mental fatigue impairs endurance cycling performance [1–3]. Recently, a study by Pires et al., [3] also found that mental fatigue reduced 20 km cycling time trial (TT_{20km}) performance by ~2.7 % and ~6.5 % when results were expressed as time and mean power output (W_{MEAN}), respectively. The authors related such an impaired cycling performance to a decreased prefrontal cortex (PFC) activation [4,5], as interpreted by the increased electroencephalography (EEG) theta wave at the 10th and 20th km of the trial. Importantly, this altered PFC activation during the TT_{20km} may have resulted from the high-demanding cognitive task performed prior to the cycling trial, as an increased PFC EEG theta wave was readily observed during the cognitive test [3]. Although the underlying mechanisms were not fully clarified, mental fatigue may have affected the higher-order cognitive control, thereby reducing the ability to deal with attentional control, encoding and storage of relevant information [6], leading to a greater perceived cost-future reward relationship and aversive sensations during exercise [2,7]. In this regard, as the exercise disengagement/investment is related to the mental representation of the sense of effort [8], manipulations capable of unbalancing the incentive-performance and effort-performance relationship (such as mental fatigue) may affect performance during exercise [2,9]. Therefore, recreational mentally fatigued cyclists may have less cognitive ability to use inhibitory control and deal with aversive sensations when regulating their pace during exercise.

Cycling trials in which a specific distance has to be covered as fast as possible are a realistic scenario that resembles conditions met in cycling training and competitions routines [3,10]. In this scenario, cyclists may base their pace on psychological responses such as the ratings of perceived exertion (RPE), as it has been suggested that athletes avoid premature fatigue and maximize performance by using a RPE template formed from previous experiences, derived from the momentary RPE in relation to the endpoint [11,12]. Consequently, mental fatigue may be a threat for a successful cycling pacing and performance regulation, as mentally fatigued cyclists may perceive a higher than expected RPE for the same power output during the trial [13]. They may have insufficient

motivation to overcome exercise-derived aversive sensations, thereby impairing performance [3,14]. Hence, interventions capable to counteract negative mental fatigue effects on endurance performance may be helpful to improve performance, particularly in recreational athletes, as they may regularly experience mental fatigue due to their high-load aerobic training routines combined with a strict-life style that encompasses dietary restrictions, longer work-days, reduced time for recovery and restricted social life [2,3].

Mental fatigue is likely associated with an elevated ATP hydrolysis and increased adenosine concentrations in the central nervous system (CNS) [15,16]. This condition has been associated with an inhibited release of excitatory neurotransmitters (such as dopamine) which reduces arousal, spontaneous behavior and affect (i.e. pleasure) during exercise [16–18]. In this regard, a substance with the potential to counteract mental fatigue effects is caffeine (CAF), as it has been suggested that CAF increases neuronal activity and excitability of the CNS by blocking neuronal A_1 adenosine receptors [17,19]. For example, a neuroimaging study observed that CAF improved the tissue oxygen extraction and reduced the cerebral metabolic rate of oxygen consumption [20]. Consequently, one may hypothesize that CAF counteracts the mental fatigue-altered PFC activation, thereby improving cycling performance in mentally fatigued cyclists. In fact, a recent study [1] provided insightful results as mentally fatigued individuals increased their cycling time-to-exhaustion after CAF ingestion (when compared to a mental fatigue trial without CAF). However, neither PFC EEG measures during the high-demanding cognitive task nor psychological measures such as motivation, affect and emotional arousal during exercise were included, therefore inferences to a more realistic distance-based time trial scenario are still required [3].

The present study verified whether CAF improved PFC activation and TT_{20km} performance in mentally fatigued cyclists. We also verified if CAF ingestion altered psychological responses to a TT_{20km} in mentally fatigued cyclists. We hypothesized that CAF ingestion would attenuate the mental fatigue-reduced PFC activation [3], improving TT_{20km} performance of mentally fatigued cyclists.

Moreover, we expected that CAF would reduce RPE, and increase affect, motivation and emotional arousal during exercise.

MATERIALS AND METHODS

Participants

The sample size was calculated through an equation suggested elsewhere ($n = 8e^2/d^2$; n, e, and d denote the required sample size, coefficient of variation and magnitude of the treatment, respectively) [21], assuming e of 1.1 % for TT_{20km} performed by recreational cyclists [22] and a conservative d of 1.0 %, thus resulting in ~ 10 participants. However, considering a possible sample loss of ~20 %, 12 non-professional trained cyclists (means and SDs of 34.3 ± 6.2 years; 179.3 ± 5.1 cm; 77.6 ± 6.8 kg) classified as performance level 3 (means and SDs of VO_{2MAX} = 58.9 ± 6.2 $mL \cdot kg \cdot min^{-1}$; W_{PEAK} = 367.0 ± 32.5 W) according to criteria suggested elsewhere [23] volunteered to take part in this study. They had a training frequency of 4.7 ± 2.3 sessions/week (283.7 ± 138.6 km/week) and a training experience of ~ 6.5 years (competing at regional and national tournaments) when the study was conducted. They were non-smokers and free from cardiovascular, visual, auditory and cognitive disorders. Three of them were non-consumers (≤ 40 mg of CAF per day), five were occasional consumers (≤ 250 mg of CAF per day) and four were daily consumers (250 ≤ consumption ≤ 572 mg of CAF per day), according to a proposed classification [24]. Importantly, CAF has been suggested as an ergogenic aid capable of improving endurance performance, regardless of habitual caffeine consumption [25,26].They were oriented to avoid consumption of stimulant (coffee, energy drink etc.) and alcoholic beverages, as well as intense exercise for the 48 h preceding the sessions. Experimental procedures, risks, and benefits were explained before collecting their written consent form signature. The procedures were previously approved by a local Ethics Committee (Process: 63787816.1.0000.5390) and performed according to the Declaration of Helsinki.

Study Design

The design of the present study encompassed 5 sessions, as depicted in Figure 1. Firstly, cyclists performed a TT_{20km} during a preliminary session (visit 1) and those who completed the trial within 33 min, were eligible to participate in the study. This criterion was based on previous TT_{20km} studies and adopted to homogenize the sample and reduce the data variability [3,27–29]. Afterwards, eligible cyclists were familiarized with a short version (~ 5 min) of the rapid visual information processing (RVIP) test and psychological scales. Thus, after preliminary session eligible cyclists attended to more four sessions: 2) Baseline: cyclists performed a baseline TT_{20km}; 3) Mental Fatigue (MF): cyclists completed a TT_{20km} after performing a 40 min RVIP test; 4 and 5) Experimental trials: cyclists completed a TT_{20km} after ingesting either CAF (MF+CAF) or placebo (MF+PLA) before the 40 min RVIP test.

Briefly, we designed the three first sessions to provide a strong, adequate control before the investigation of CAF effects on mentally fatigued cyclists. Thus, sessions 1 (preliminary TT_{20km}) and 2 (baseline TT_{20km}) were designed to provide TT_{20km} performance measures reliability. Moreover, despite most studies have consistently shown that mental fatigue impairs endurance performance [13], only one showed mental fatigue effects on TT_{20km} [3]. Hence, rather than assuming it, we confirmed that mental fatigue impaired TT_{20km} performance (a proof-of-concept) by comparing session 2 vs session 3 (baseline TT_{20km} vs MF TT_{20km}). Consequently, sessions 4 and 5 were designed to investigate if CAF may improve TT_{20km} performance in mentally fatigued cyclists, so that MF+CAF and MF+PLA trials were performed in a double-blinded, counterbalanced order. The study was finished within 30 days, the sessions were interspersed by a 3-7 days washout period, performed at the same time of the day, under controlled temperature (~24◦C) and humidity (50–60%). Psychological responses such as RPE, motivation, emotional arousal and affect were measured every 2 km through the TT_{20km}, while EEG, motivation, emotional arousal, and mental fatigue sensation were also obtained before and after the RVIP test. After the study conclusion, cyclists performed a maximal graded test (25 $W \cdot min^{-1}$ increments until voluntary exhaustion) in order to obtain their VO_{2MAX} and peak power output (W_{PEAK}).

*** Figure 1 ***

Mental Fatigue Protocol

The RVIP test was performed in a silent and illuminated room [30]. Cyclists sat comfortably on a chair, frontally to a 17 inches colored monitor, and wore an earphone damper to avoid noise distractions. The RVIP test consisted of a 40 min high-demanding cognitive task, which randomly displayed single numbers (numbers from 1 to 9 being displayed isolated) in a white box in the center of the monitor, in a rate of 100 numbers per minute (one number per 600 milliseconds). They were asked to press the space bar of a standard keyboard every time they identified a sequence of three even (e.g., 2, 4, 6; 4, 6, 8 etc.) or odd numbers (e.g., 3, 5, 7; 3, 9, 7 etc.), shown eight times a minute. Cognitive performance was measured as false alarms (expressed as arbitrary units; a.u.), reaction time (s) and percentage of accuracy answers (i.e. correct numerical sequences %).

Caffeine and Placebo Ingestion

We followed the recommendations of the International Society of Sports Nutrition (ISSN) position for CAF ingestion [24]. Briefly, it has been suggested that 3 to 6 $mg \cdot kg^{-1}$ of body mass of CAF significantly improve endurance performance in trained athletes approximately 1 h post-ingestion [24]. In order to accomplish this recommendation, participants ingested 5 $mg \cdot kg^{-1}$ of body mass of CAF or PLA immediately before the RVIP test (~ 50 min before the cycling TT_{20km} commencement). The CAF and PLA capsules were formulated to have the same appearance (i.e. form, size and color) and contain the same taste and smell, thereby ensuring that cyclists could not identify differences between them. CAF was manipulated as previously reported in a mental fatigue-caffeine study [1]. In contrast, PLA was manipulated in cellulose capsules containing inert substances such as a lubricant, magnesium stearate, and magnesium silicate. Importantly, neither participants nor researchers appointed to the data collection were aware of the intervention. Likewise, researchers

appointed to data analysis were blinded to manipulations, thus characterizing the present study as a truly double-blind study.

Cycling Time Trial (TT_{20km})

Cyclists performed the baseline, MF, MF+CAF and MF+PLA TT_{20km} having only distance as available feedback, that is they were blinded to feedback such as time, cadence, speed, power output, and heart rate. Cyclists used a road bicycle (Giant®, New York, USA) attached to a cycle simulator (Computrainer, Racer Mate ® 8000, Seattle, USA) that provided power output (W), cadence (rpm) and speed ($km \cdot h^{-1}$) data throughout the trials. The device was calibrated before each test according to the manufacturer's instructions. The bike was individually adjusted according to cyclists' preferences and they were allowed to drink water *ad libitum* during the trials. The time to complete the TT_{20km} and the W_{MEAN} recorded throughout the trial were used as performance measures. Furthermore, power output data were averaged every 2 km to analyze pacing strategy.

Measures and Instruments

Electroencephalography (EEG)

Previous EEG studies have suggested that EEG theta wave is a slow frequency EEG band sensitive to distinguish a mental fatigue state [3–5]. Additionally, theta rather than alpha wave may be a reliable distinguisher of changes in cognitive processing as mental fatigue progresses, as frontal cortex EEG theta wave is correlated with the percentage of accuracy answers (i.e. error rate) during high-demanding cognitive tasks [5]. Hence, PFC activation was continuously obtained through an EEG unit (Emsa®, EEG BNT 36, TiEEG, Brazil) at the Fp1 position, according to the international EEG 10-20 system [31]. This position was ensured according to frontal and sagittal planes, referenced to mastoid. The EEG was recorded at a 600 Hz sampling frequency, through active electrodes (Ag-AgCl) with resistance ~5 KΩ. After exfoliation and cleaning, electrodes were fixed with a conductive gel, adhesive tape, and medical strips. The EEG signal was recorded during 3 min rest, immediately

before and after the RVIP test, when participants were completely calm, maintaining their eyes closed and avoiding head and trunk movements.

The EEG signal was amplified (gain of $1 \cdot 10^3$) and filtered with a digital notch (60 Hz), thereafter a 1-30 Hz bandpass filter was applied. EEG signal showing spectral leakage, defined as a signal $\geq$ 100 μv, were considered as artifacts (n = 1-2, depending on the moment of the experimental setup) and were excluded from the analysis [31]. Furthermore, data recorded during the first and last 30 s of a 180 s time window were removed to avoid eventual noise associated with the participants' movements when expecting the EEG record start and stop. Thereafter, EEG data were analyzed in frequency domains through a fast-Fourier transformation so that the total power spectral density (tPSD) of the theta wave (3 – 7 Hz) was calculated over the most steady (i.e. lowest SD) 30 s window (determined through an algorithm implemented in Matlab® environment).

Psychological Responses

Responses of mental fatigue sensation, emotional arousal and motivation were obtained before and immediately after the RVIP test. Briefly, the mental fatigue sensation was rated through a 100 mm visual analogue scale (VAS), then cyclists were required to answer "How mentally fatigued you feel now?" having 0 (zero) as "none at all" and 100 as "maximal" mental fatigue, as reported elsewhere [32]. The emotional arousal was assessed through a 6-points felt arousal scale (FAS) that ranks the emotional arousal within categories ranging from "low activation" to "high activation" [33]. The perception of high emotional arousal may be interpreted as a state of "worked-up" whilst perception of low emotional arousal, as a state of "relaxation". Moreover, motivation was assessed through a 10 points Likert scale having two opposite motivational descriptors, that is 0 (zero) as "not all motivated" and 10 as "extremely motivated" [34,35]. These responses, expressed as arbitrary units (a.u.), were compared between pre and post RVIP test.

Furthermore, emotional arousal, motivation, affect and RPE responses were obtained every 2 km of the TT_{20km}. Affect responses (pleasure/displeasure) were obtained by using the 11-points

feeling scale (FS), as suggested elsewhere [36]. This single-item bipolar scale (-5 to +5) uses descriptors as "neutral" (zero), "very good" (+5) and "very bad" (-5) to rate the affective valence. Furthermore, RPE was obtained through a 15-points Borg scale, as suggested elsewhere [37]. In order to verify possible mental fatigue-induced psychological alterations during exercise, motivation, FAS, FS and RPE (expressed as a.u.) were analyzed every 2 km. Given the comparable absolute RPE responses in control and mental fatigue, although the reduced power output values under mental fatigue [3], we also calculated the RPE-power output ratio (RPE_W) for every 2 km of the TT_{20km}.

Statistical Analysis

Gaussian distribution and homoscedasticity were previously checked through Shapiro-Wilk and Levene tests, respectively, and results were reported as mean and standard deviation (± SD).

Firstly, we checked the reliability on performance measures by comparing preliminary TT_{20km} (session 1) and baseline TT_{20km} (session 2), and reporting the typical error of measurement (expressed as a variation of the grand mean) and the correlation coefficient between them [21].

Secondly, as a proof-of-concept of mental fatigue effects we verified if performance in MF TT_{20km} was impaired when compared to baseline TT_{20km}. Therefore, time and W_{MEAN} responses in baseline and FM TT_{20km} were compared through a paired T-student test (session 2 vs session 3). Particularly in MF session (session 3), we also compared pre to post RVIP test alterations in EEG theta power, VAS, FAS and motivation through a paired T-student test.

Effects of CAF ingestion on mentally fatigued cyclists were assessed in different ways. Firstly, to mitigate the impact of inter-individual variability (between sessions) we expressed EEG and psychological responses (i.e. sensation of fatigue, emotional arousal, and motivation) as Δ values from pre-treatment (pre to post RVIP measures) and compared MF+CAF and MF+PLA responses through a paired T-student test. Secondly, we compared cognitive performance (i.e. false alarms, reaction time and accuracy of answers averaged during the RVIP test) between MF+CAF and MF+PLA sessions through a paired T-student test.

Furthermore, we confirmed if CAF improved TT_{20km} performance (W_{MEAN} and time) in mentally fatigued cyclists. Accordingly, to mitigate the impact of inter-individual variability we expressed TT_{20km} performance as Δ values from MF TT_{20km}, and compared MF+CAF and MF+PLA through a paired T-student test. In addition, we analyzed pacing (i.e. power output) and psychological responses (i.e., RPE, RPE_W, motivation, FAS and FS) during the MF+CAF and MF+PLA through a 10 x 2 mixed model having distance (2nd, 4th up to 20th km) and condition (MF+CAF *vs* MF+PLA) as fixed factors, and cyclists as the random one. The AIC index (Akaike's information criterion) determined the covariance matrix that best fitted to the dataset (Compound Symmetric, First-order Autoregressive homogeneous and heterogeneous, First-order Autoregressive Moving Average, and Toeplitz homogeneous and heterogeneous), and the Bonferroni test corrected p values in multiple comparisons.

We reported the post-hoc ES analysis (expressed as *d*-Cohen) to make eventual comparisons with previous studies possible [3,28], so that ES was interpreted as small (< 0.2), moderate (0.2 to 0.6), large (0.6 to 1.2), very large (1.2 to 2.0), and extremely large (≥ 2.0), as suggested elsewhere [38]. Results were significant when $p \leq 0.05$.

RESULTS

As part of the study control, we checked the reliability of performance measures. There was no difference in time ($p = 0.81$; $d = 0.074$, small ES) and W_{MEAN} ($p = 0.27$; $d = 0.066$, small ES) between preliminary (32.8 ± 1.3 min and 262.3 ± 37.5 W) and baseline (32.7 ± 1.4 min and 260.0 ± 32.0 W) sessions. The typical error of measurement and correlation between preliminary (trial 1) and baseline (trial 2) sessions were 0.8 % and $r = 0.94$, and 3.1 % and $r = 0.96$ for time to complete the trial and W_{MEAN}, respectively.

Proof-of-Concept of Mental Fatigue Effects

As a proof-of-concept, we verified if TT_{20km} performance was impaired by mental fatigue, given the 0.9 ± 0.7 % increase in time to complete the trial (32.7 ± 1.4 min vs 33.0 ± 1.4 min; p = 0.00; *d* = 2.41, extremely large ES) and the 2.2 ± 1.6 % reduction in W_{MEAN} (260 ± 32 W vs 254.3 ± 29.7 W; p = 0.00; *d* = 2.87, extremely large ES) in mental fatigue trial when compared to baseline. Figure 2 depicts the percentage of alteration in time (panel A) and power output (panel B) from baseline TT_{20km}.

Furthermore, we observed that the RVIP test changed PFC activation in MF session, as we observed a ~ 4.8 ± 7.1 % increase in EEG theta band from pre to post RVIP test (p = 0.03; *d* = 1.53, very large ES). Accordingly, cyclists rated increased mental fatigue sensation (35.0 ± 16.9 vs 73.3 ± 12.1 a.u.; p = 0.000; *d* = 3.40, extremely large ES), reduced motivation (7.6 ± 1.9 vs 6.0 ± 2.9 a.u.; p = 0.009; *d* = 3.17, extremely large ES) and lower emotional arousal (4.7 ± 1.4 vs 3.8 ± 1.5 a.u.; p = 0.002 *d* = 2.73, extremely large ES) when comparing pre to post RVIP test responses. Mentally fatigued cyclists showed a reaction time of 37.0 ± 12.4 s, false alarms of 22.4 ± 17.4 and accuracy of 41.8 ± 16.1% during the RVIP test.

*** Figure 2 ***

C*affeine Effects on EEG, Psychological and Cognitive Performance Responses in Mentally Fatigued Cyclists*

In contrast to the ~ 4.8 ± 7.1 % increase in EEG theta wave found in MF condition, we observed a ~ 8.8 ± 13.9 % and ~ 4.8 ± 17.9 % reduction in EEG theta wave from pre to post RVIP test in MF+CAF and MF+PLA sessions, respectively (Figure 2). Importantly, the Δ alteration in PFC activation was comparable between MF+CAF and MF+PLA sessions (p = 0.25; *d* = 0.50, moderate ES).

Regarding the RVIP test-induced psychological alterations, mental fatigue sensation increased from pre to post RVIP test in both MF+CAF (↑ 65.7 ± 105.4%) and MF+PLA sessions (↑ 114.8 ± 113.0%), but the Δ alteration was significantly higher in MF+PLA than in MF+CAF (p = 0.02; *d* = 0.70, large ES). In contrast, there was an increase in emotional arousal in MF+CAF (↑ 11.4 ± 15.8%) but a decrease in MF+PLA (↓ 18.1 ± 24.2%), thus Δ alteration from pre to post RVIP test was significantly different between conditions (p = 0.01; *d* = 1.51, very large ES). Furthermore, motivation changed slightly from pre to post RVIP test in MF+CAF (↓ 4.6 ± 15.5%) and MF+PLA (↑ 3.3 ± 31.7%), therefore no significant Δ alterations were observed between conditions (p = 0.67; *d* = 0.15, small ES).

Comparable cognitive performance was observed between MF+CAF and MF+PLA, as Δ alterations of reaction time (38.0 ± 14.5 s *vs* 39.8 ± 13.8 s, respectively; p = 0.39; *d* = 0.13, small ES), false alarms (19.7 ± 18.1 *vs* 13.4 ± 11.2, respectively; p = 0.23; *d* = 0.42, moderate ES) and accuracy (46.4 ± 16.1% *vs* 46.8 ± 17.3%, respectively; p = 0.83; *d* = 0.024, small ES) were not significantly different between conditions.

Caffeine Effects on TT_{20km} Performance and Pacing in Mentally Fatigued Cyclists

Mentally fatigued cyclists significantly improved TT_{20km} performance in CAF when compared to PLA ingestion. The 1.8 ± 1.4 % improvement in time to complete the trial with CAF ingestion (32.4 ± 1.2 min) was significantly greater (p = 0.002, *d* = 2.36, extremely large) than the 0.09 ± 1.5 % improvement with PLA ingestion (33.0 ± 1.2 min). Accordingly, the 4.8 ± 4.1 % improvement in W_{MEAN} in MF+CAF (265.8 ± 28.2 W) was significantly greater (p = 0.001, *d* = 2.72, extremely large ES) than the 0.7 ± 3.9 % improvement in MF+PLA session (256.0 ± 25.3 W).

Cyclists adopted a similar "J-shaped" pacing profile throughout the MF+CAF and MF+PLA trials. Multiple comparisons revealed a condition (*F* = 11.62, p = 0.005, *d*= 1.45, very large ES) and a distance main effect (*F* = 17.49, p = 0.000, *d* = 1.78, very large ES) in power output, despite no condition by distance interaction effect was observed (*F* = 0.28, p = 0.97, *d* = 0.23, small ES). Figure

3 (panels A and B) showed performance Δ values from MF TT_{20km} while figure 4 depicted pacing responses.

Figure 3

***Figure 4 ***

Caffeine Effects on TT_{20km} Psychological Responses in Mentally Fatigued Cyclists

Comparable results were observed in absolute RPE values, as neither a condition main effect (F = 2.24; p = 0.16; d = 0.63, very large ES) nor a condition by distance interaction effect (F = 1.18; p = 0.33; d = 0.46, large ES) was detected, despite the distance main effect in absolute RPE values (F = 12.27, p = 0.000, d = 1.43 extremely large ES). However, there was a significant condition main effect (F = 10.32; p = 0.005, d = 1.37, extremely large ES) as well as a distance main effect (F = 4.28, p = 0.001, d = 0.82, large ES) in RPE_W data, as the increase in RPE_W during the TT_{20km} was lower in CAF than in PLA. However, no condition by distance interaction effect was found in RPE_W (F = 1.29, p = 0.278, d = 0.48, large ES). Overall RPE responses were shown in Figure 5 (panel A and B).

***Figure 5 ***

Regarding the remaining psychological responses, a condition main effect (F = 5.72, p = 0.018, d = 1.02 large ES) and a distance by condition interaction effect (F = 2.29, p = 0.019, d = 0.65 large ES) was found in affect, as cyclists reported higher affect in MF+CAF than in MF+PLA when they were spurting at the end of the trial (p = 0.000). However, no distance main effect was detected in affective valence (F = 1.47, p = 0.169, d = 0.52 moderate ES). In contrast, neither a distance main effect (F = 0.45, p = 0.90, d = 0.29 moderate ES) nor a distance by condition interaction effect (F = 0.87, p = 0.55, d = 0.40 moderate ES) was observed in motivation. Nevertheless, a condition main effect (F = 4.61, p = 0.033, d = 0.92, large ES) was observed so that motivation was greater in mentally

fatigued cyclists after in MF+CAF trial. Accordingly, although neither a distance main effect (F = 0.78, p = 0.64, d = 0.38 moderate ES) nor a distance by condition interaction effect (F = 0.88, p = 0.54, d = 0.40 moderate ES) was found in emotional arousal. However, mentally fatigued cyclists rated higher arousal throughout the TT_{20km} in CAF than PLA (F = 11.03, p = 0.001, d = 1.42 very large ES).

***Figure 6 ***

DISCUSSION

The present study was designed to investigate if CAF ingestion may revert mental fatigue effects on PFC activation, thus improving cycling time trial performance in mentally fatigued recreational cyclists. Results showed that CAF reverted the mental fatigue-reduced TT_{20km} performance, despite the comparable CAF and PLA effects on PFC activation. Additionally, CAF reduced RPE and changed other psychological responses throughout the trial. Then, results suggest that CAF is capable to revert the mental fatigue-reduced cycling time trial performance, but challenged its role in cortical activation.

Proof-of-concept of mental fatigue effects on cycling performance

Most studies have shown that mental fatigue impairs endurance performance , but only one showed that mental fatigue impaired TT_{20km} performance [3]. This study suggested that the reduced TT_{20km} performance was possibly related to a mental fatigue-reduced PFC activation. Hence, as a proof-of-concept, firstly we confirmed that mental fatigue affected PFC activation and TT_{20km} performance. We found a change in PFC activation after the RVIP test, indicated by the increased slow-frequency EEG band suggested to distinguish mental fatigue states [3–5]. Moreover, cyclists rated a higher fatigue sensation and lower motivation and emotional arousal after this high-demanding cognitive task. Accordingly, when comparing baseline and MF trials we observed that mental fatigue

reduced cycling performance outcomes after the reliability measures have evidenced that performance was steady (i.e. no learning or training effects from preliminary to baseline trial). Thus, together with others [3], this part of the present study reinforced the notion of a likely connection between PFC activation and impaired TT_{20km} performance. Briefly, it has been proposed that TT_{20km} is a self-paced exercise that requires superior inhibitory control and ability to deal with aversive sensations [2,9], and that PFC is involved in proactive, goal-directed behavior [3,26,39,40]. Therefore, although we have not measured PFC activation during TT_{20km} we found an altered PFC activation readily after the RVIP test, showing that PFC activation may have played a role on TT_{20km} performance in mentally fatigued cyclists [3].

Caffeine effects on high-demanding cognitive task responses

Although the mechanism underlying mental fatigue effects is not fully understood, the reduced PFC activation could be a result of an enhanced cerebral ATP hydrolysis-derived adenosine concentration during cognitive overload [15,16]. Then, we had also hypothesized that CAF ingestion may counteract mental fatigue effects as CAF blocks neuronal A_1 adenosine receptors and improves the neuronal activity and excitability of the CNS [17,19]. However, we observed that both CAF and PLA similarly increased the PFC activation when expressed as pre-to-post RVIP changes. Accordingly, a recent study also reported similar cortical changes to CAF and CAF-perceived PLA ingestion, thereby challenging the effects of CAF ingestion on cortical activation [29]. It has been proposed that the expectation of receiving a given substance (such as CAF) during a PLA ingestion may induce cortical changes in the direction of the active substance [29,41]. Thus, perhaps the cyclists may have experienced some PLA effects as reported elsewhere [29], although we have used a true double-blind design in the present study. Therefore, although knowing that they had 50% chance of ingesting CAF or PLA in each experimental session, the uncertainty about the substance ingested may have led them to expect some CAF effects. However, PLA effects in laboratory settings have been poorly understood and require future studies.

Interestingly, both CAF and PLA also improved cognitive performance responses to the RVIP test. However, CAF attenuated the mental fatigue-induced negative sensations rather than PLA, as indicated by the lower fatigue sensation and higher emotional arousal in CAF than in PLA after the RVIP test. Somehow, the cycling performance in MF+CAF trial may have benefited from an alleviated mental fatigue-induced negative sensations before starting the TT_{20km}.

Caffeine effects on TT_{20km} performance and psychological responses in mentally fatigued cyclists

Actually, regardless of a "J-shaped" pacing strategy adopted in all experimental sessions, CAF ingestion improved TT_{20km} performance expressed as time and W_{MEAN} when compared to PLA. Interestingly, mentally fatigued cyclists showed an "improved psychological state" after CAF ingestion, given the reduced RPE_W ratio, and increased affect, motivation and emotional arousal during TT_{20km}.

It has been proposed that a successful distance-based cycling trial performance such as a TT_{20km}, is related to the cognitive ability to preserve inhibitory control while dealing with aversive sensations [2,9], because cyclists would be required to adequately evaluate the perceived cost-future reward relationship during exercise in order to maximize their pace and complete the trial as fast as possible. Consequently, mental fatigue is considered as a threat to a successful TT_{20km} performance. When compared to PLA, CAF reduced the mental fatigue-negative sensations after the RVIP test. Likewise, mentally fatigued cyclists completed the TT_{20km} reporting lower RPE_W, higher affective valence, motivation and emotional arousal after CAF ingestion. Perhaps, CAF prevented cyclists from the RVIP test-induced cognitive depletion before the trial, thereby allowing them to complete the TT_{20km} under an "improved psychological state" when compared to PLA [2]. Somehow, these improved psychological responses are likely associated with an improved cycling performance as reported elsewhere [1].

Methodological aspects and practical implications

CAF has been suggested as a powerful aid in improving endurance performance regardless of habitual caffeine consumption [25], mainly through its action on the CNS [1,19,42]. It should be pointed out that peripheral effects such as an increased glycolytic flux, mitochondrial oxidation rate and lipid oxidation-induced muscle glycogen sparing [42] have been reported in millimolar doses (supra-physiological) of CAF [19,43]. Therefore, considering that we used oral doses ~ 100 times lower than millimolar dosage [43], it is unlikely that mentally fatigued cyclists have improved cycling performance due to a peripheral CAF action [44].

Recently, a study showed that CAF reverted negative mental fatigue effects on cycling time-to-exhaustion performance [1] while another verified that mental fatigue impaired TT_{20km} performance [3]. Thus, we combined both hypotheses, as cyclists may experience mental fatigue and use supplements as CAF in training and competitions. In fact, a study by Stewart et al., [45] verified that cyclists committed to the sport may perceive pressure to use supplements to improve performance. In contrast, cyclists may experience mental fatigue due to the high-load aerobic training routines combined with a strict-life style in daily activities [2,3]. Thus, our results have practical implications as we verified that CAF counteracted the mental fatigue-reduced performance during a cycling trial that resembles the conditions met in cycling competitions and training sessions [10,27].

In order to potentiate our manipulation we administered CAF readily before the RVIP test. Because the oral CAF ingestion has a ~ 45-60 min time course [24] cyclists had to ingest CAF immediately before the 40 min RVIP test, as the ingestion after the RVIP test could have missed some mental fatigue effects. Despite most effects likely occurring from 45-60 min after CAF ingestion, we cannot ensure that cyclists did not experience some CAF effects during the RVIP. Actually, cyclists reported attenuated psychological changes readily after the RVIP test when they ingested CAF. Future studies may verify if other soluble central-action compounds or tasting CAF (instead of ingesting) may also counteract mental fatigue effects on performance.

CONCLUSIONS

The present study showed that CAF improved TT_{20km} performance in mentally fatigued cyclists, regardless of alterations in PFC activation. Furthermore, CAF ingestion attenuated the mental fatigue-induced negative sensations, thus reducing RPE and increasing affect and emotional arousal during the cycling trial.

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FIGURES' CAPTION

Figure 1. Schematic representation of the study design. TT_{20km} is 20 km cycling time trial. MF is mental fatigue; CAF is caffeine; PLA is placebo; RVIP is rapid visual information processing; EEG is electroencephalography.

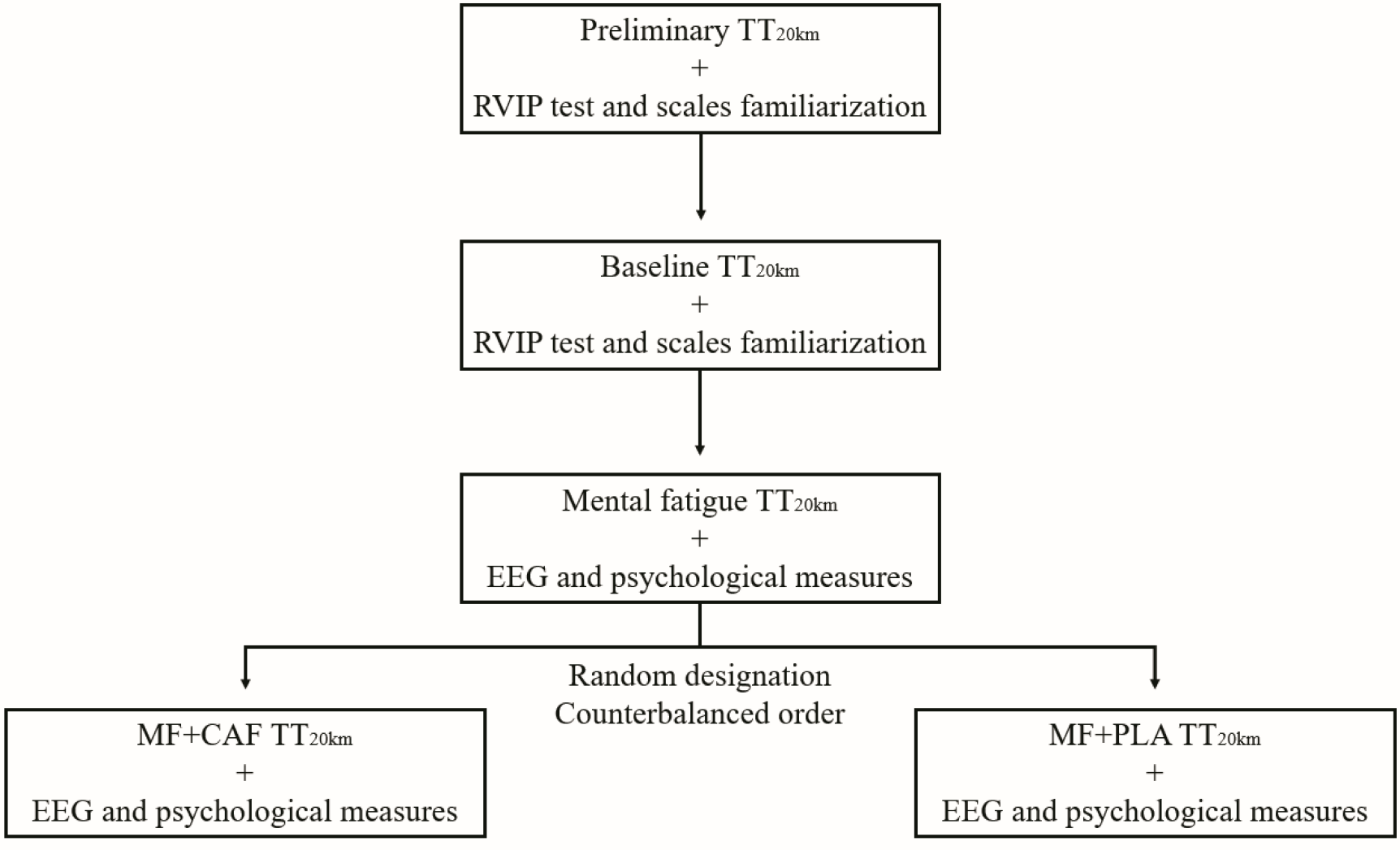
Figure 2. Performance changes from baseline to mental fatigue trial expressed as time to complete the TT_{20km} (panel A) and W_{MEAN} (panel B).

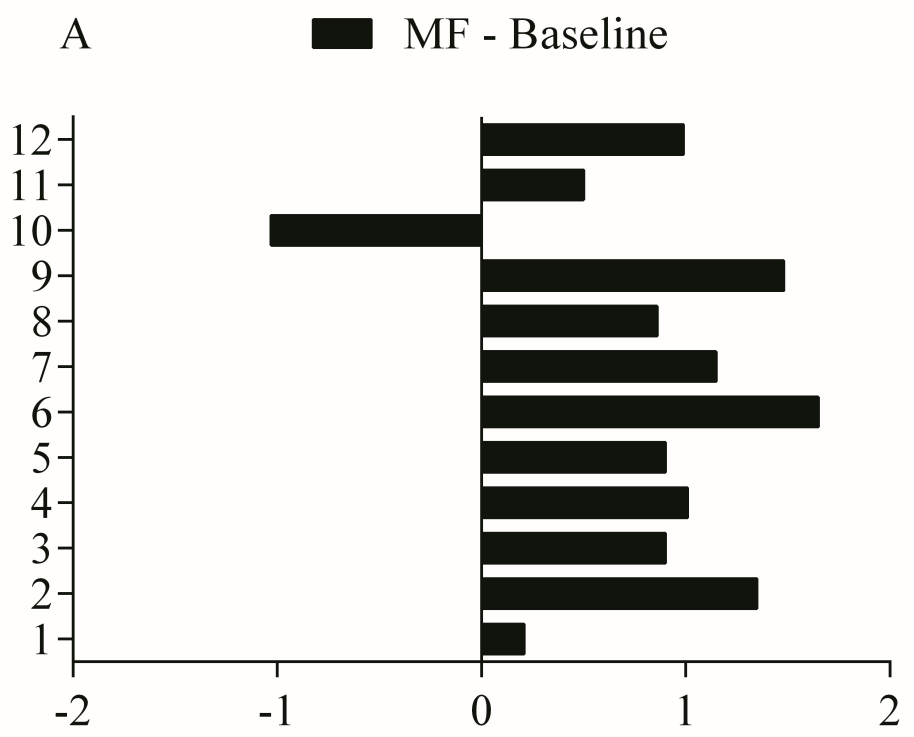
Figure 3. Performance changes in mentally fatigued cyclists after caffeine (MF+CAF) and placebo (MF+PLA) ingestion. Data of time to complete the TT_{20km} (panel A) and W_{MEAN} (panel B) were reported as mean ± SD. * indicates that time to complete TT_{20km} ($p = 0.002$) and W_{MEAN} ($p = 0.001$) were significantly different.

Figure 4. Power output responses of mentally fatigued cyclists throughout the TT_{20km} after caffeine (MF+CAF, filled circles) and placebo (MF+PLA, open circles) ingestion. # is condition main effect ($p = 0.005$); * is distance main effect ($p = 0.000$). Data were reported as mean ± SD.

Figure 5. Absolute RPE (panel A) and RPE_W (panel B) values in mentally fatigued cyclists throughout the TT_{20km} after caffeine (MF+CAF, filled circles) and placebo (MF+PLA, open circles) ingestion. # is condition main effect for RPE_W ($p = 0.005$); * is distance main effect for RPE ($p = 0.000$) and RPE_W ($p = 0.001$). Data were reported as mean ± SD.

Figure 6. Psychological responses in mentally fatigued cyclists throughout the TT_{20km} after caffeine (MF+CAF, filled circles) and placebo (MF+PLA, open circles) ingestion. # is a condition main effect in affective valence ($p = 0.018$), motivation ($p = 0.033$) and emotional arousal ($p = 0.001$); Condition by distance interaction effects are shown in boxes. Data were reported as mean ± SD.


Preliminary TT20km
+
RVIP test and scales familiarization
Baseline TT20km
+
RVIP test and scales familiarization
Mental fatigue TT20km
+
EEG and psychological measures
Random designation
Counterbalanced order
MF+CAF TT20km
+
EEG and psychological measures
MF+PLA TT20km
+
EEG and psychological measures


A
MF - Baseline
12
11
10
9
8
7
6
5
4
3
2
1
-2
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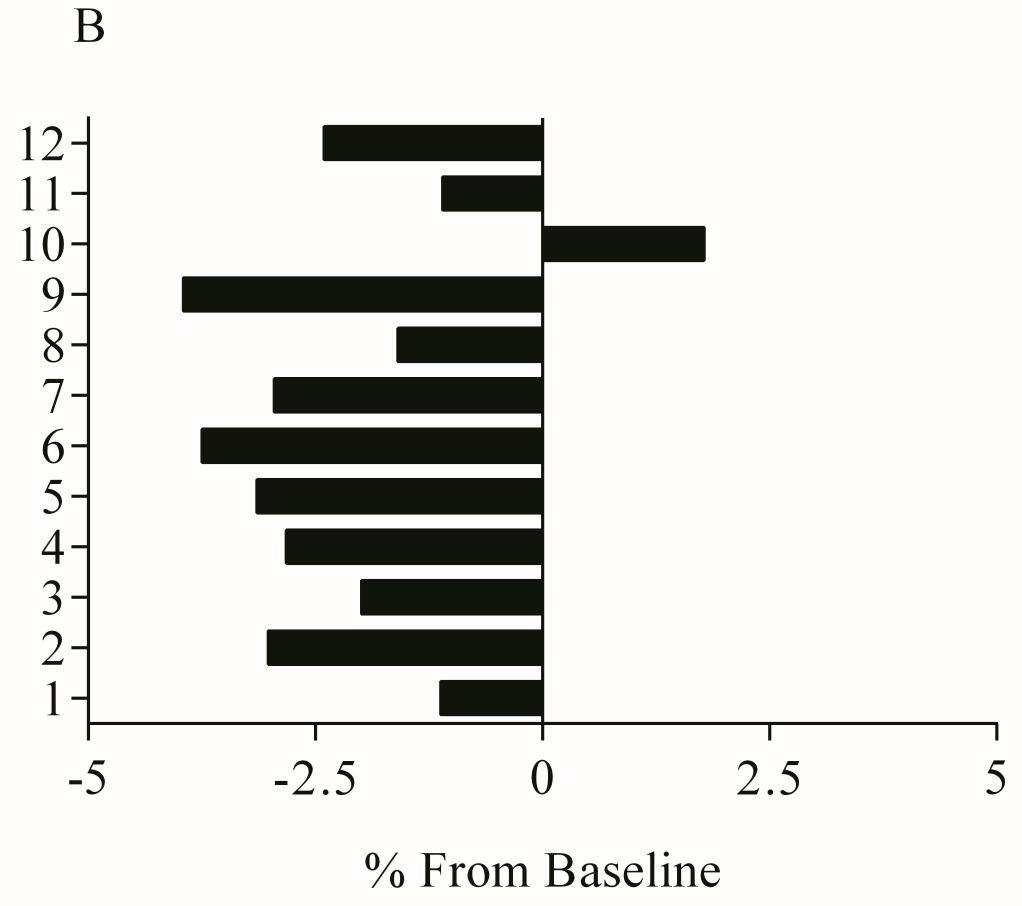
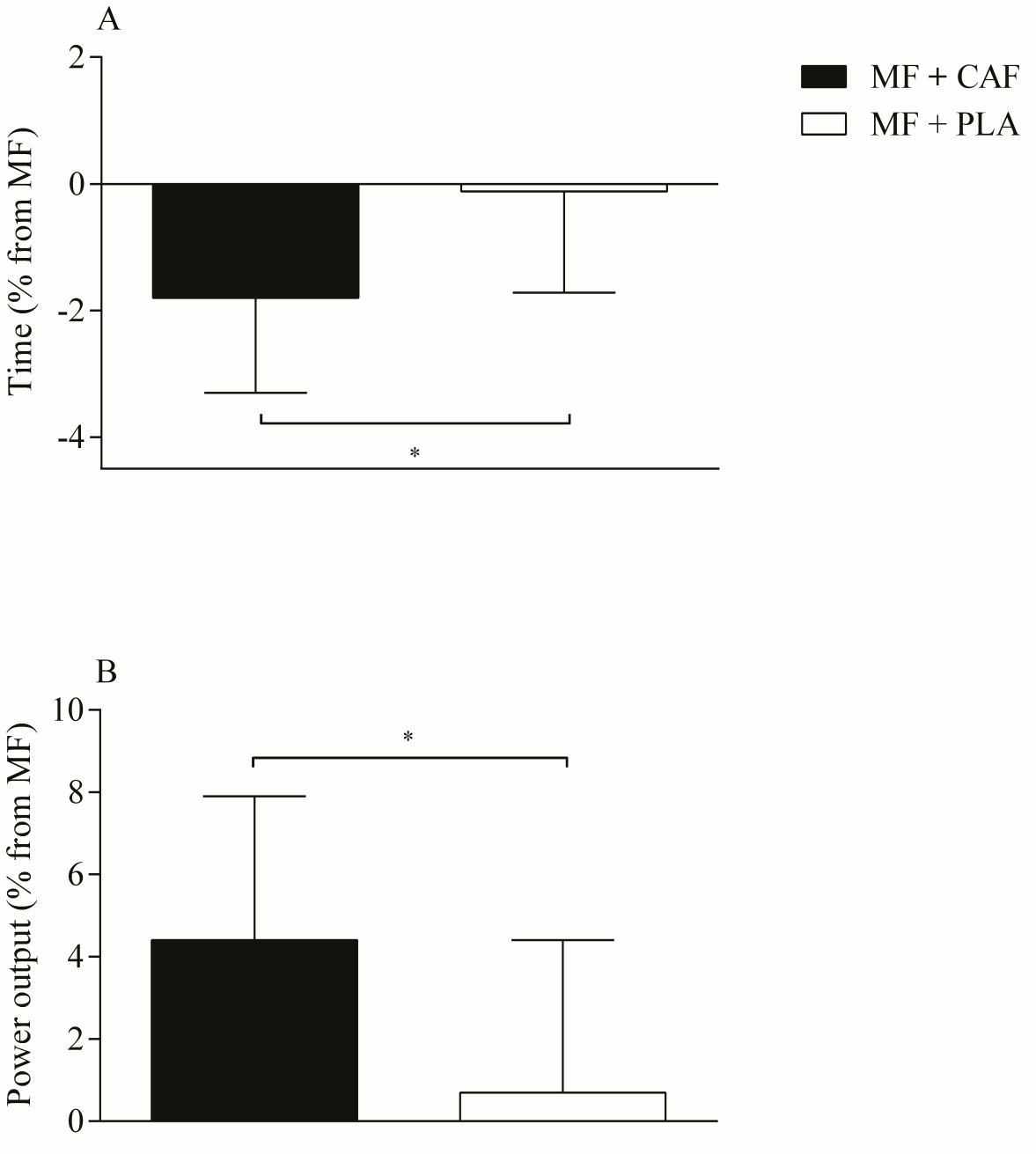
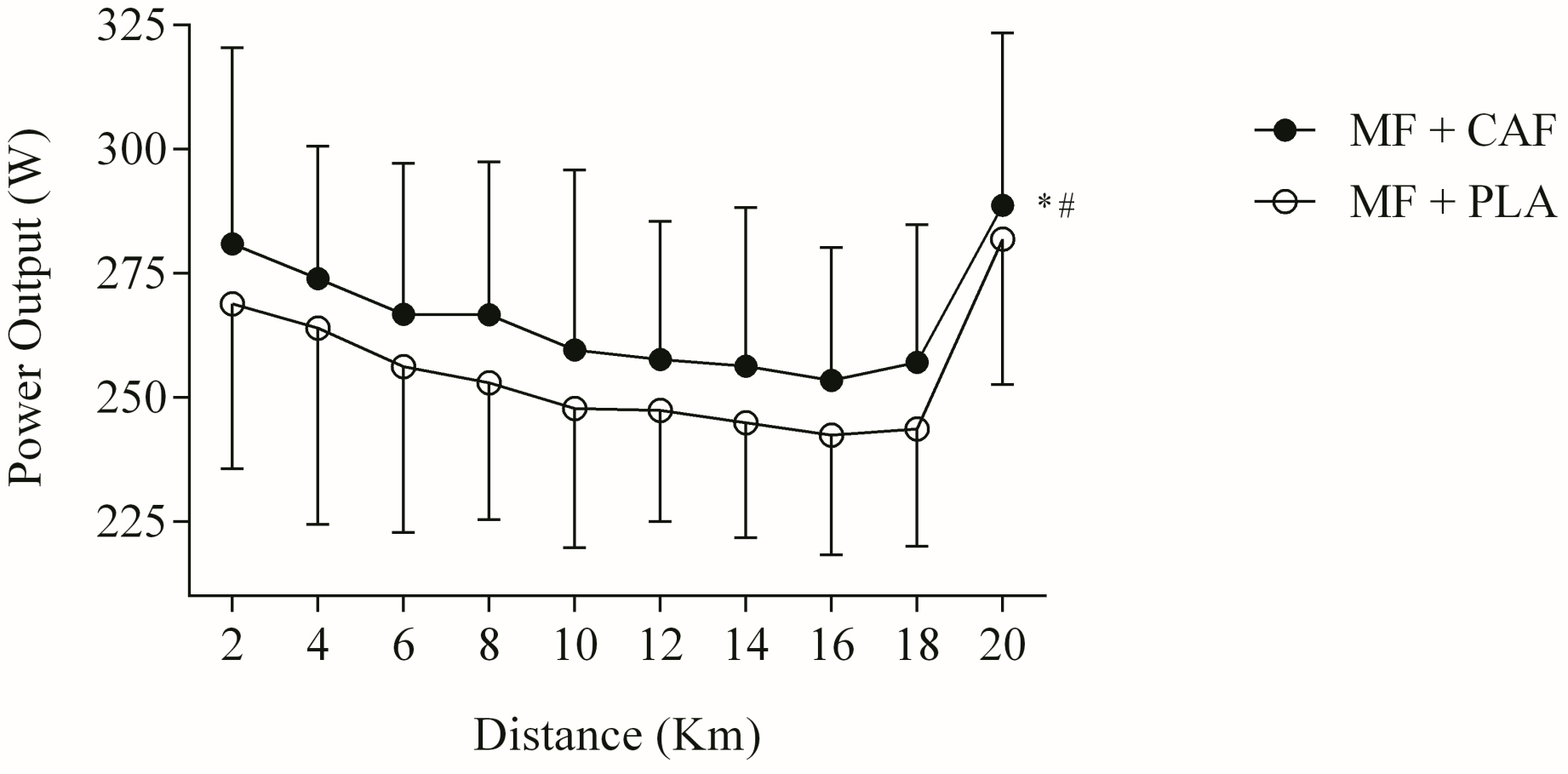
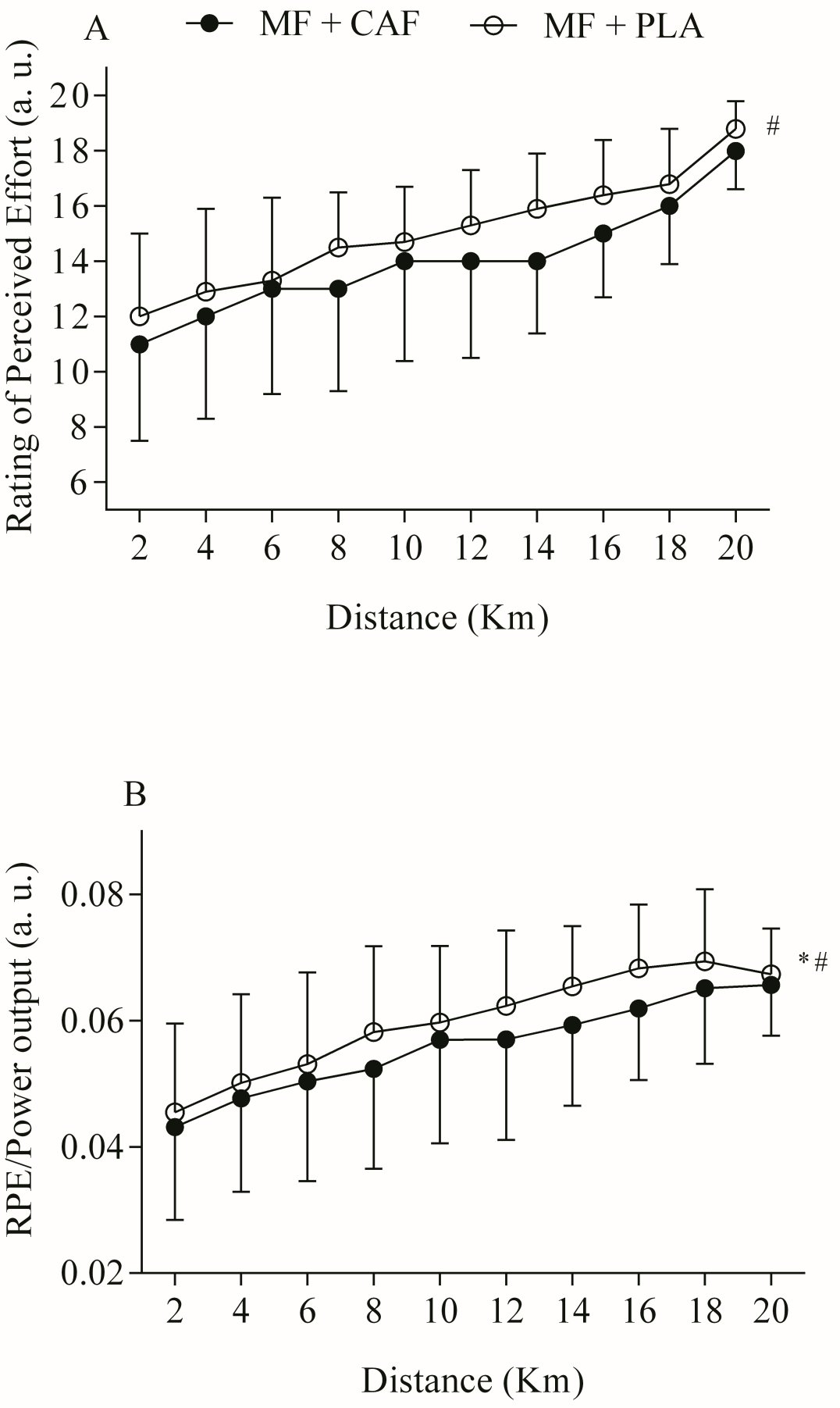
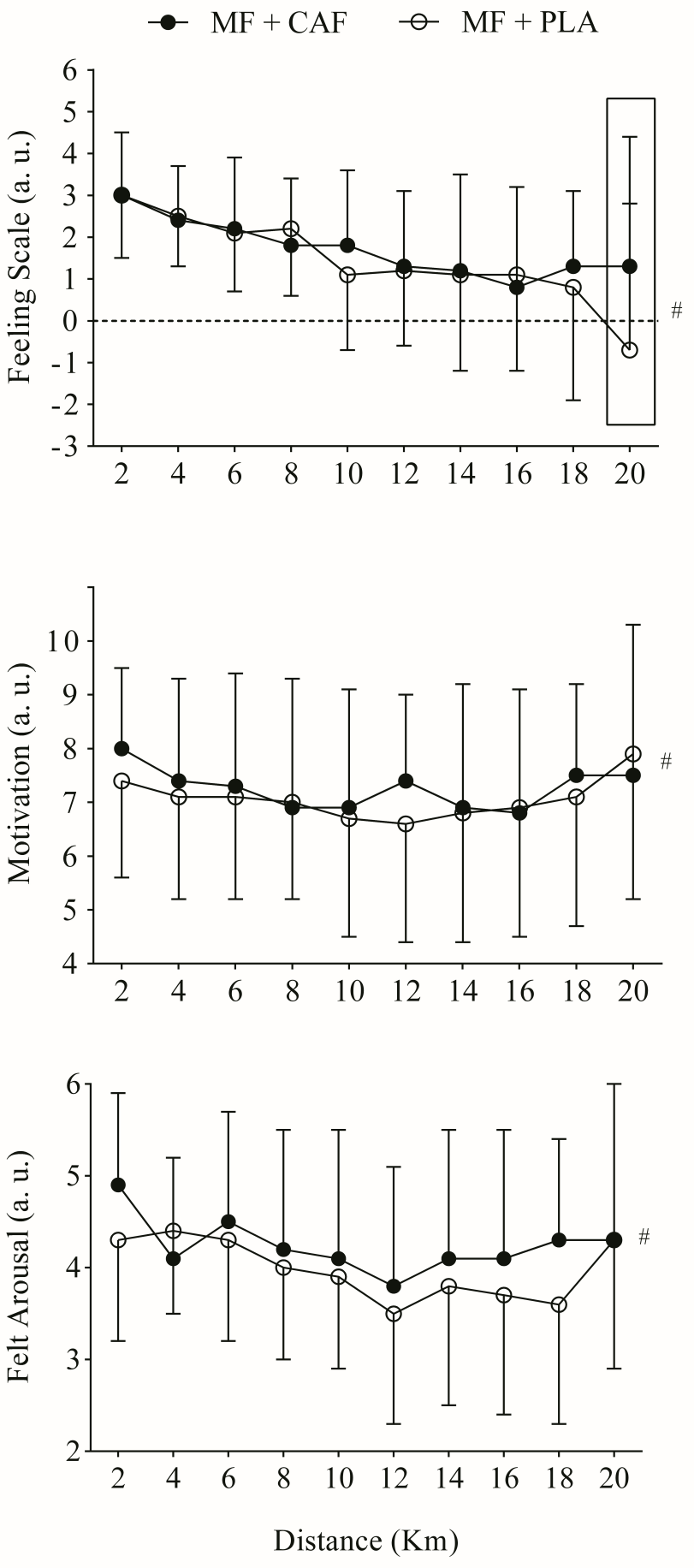
0
1
2


B
12
11
10
9
8
7
6
5
4
3
2
1
-5
-2.5
0
2.5
5
% From Baseline


A
2
0
-2
-4
Time (% from MF)
*
MF + CAF
MF + PLA
B
10
8
6
4
2
0
Power output (% from MF)
*


325
300
275
250
225
Power Output (W)
2
4
6
8
10
12
14
16
18
20
Distance (Km)
*#
MF + CAF
MF + PLA


A
MF + CAF
MF + PLA
Rating of Perceived Effort (a. u.)
20
18
16
14
12
10
8
6
#
2
4
6
8
10
12
14
16
18
20
Distance (Km)
B
RPE/Power output (a. u.)
0.08
0.06
0.04
0.02
*#
2
4
6
8
10
12
14
16
18
20
Distance (Km)


MF + CAF
MF + PLA
Feeling Scale (a. u.)
Motivation (a. u.)
Felt Arousal (a. u.)
Distance (Km)
#